STATISTICAL ANALYSIS PLAN (SAP)

Association between surrogate markers of inflammation as detected by ultrasound and results of an intensive weight loss program obese patients with knee osteoarthritis: a prospective cohort study based on the CAROT Trial

v.2.0 (January 17, 2017)

Collaborators/authors:

B. F. Riecke[1], Sabrina M. Nielsen[1], R. Christensen[1], A. F. Riecke[1], P. Christensen[1,2], H. Gudbergsen[1], S. Torp-Pedersen[1], M. Boesen[1,3], E.M. Bartels[1], M. Henriksen[1,4], H. Bliddal[1]

1: The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

2: Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Denmark.

3: Department of Radiology, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark

4: Department of Therapy, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark

Correspondence to:

Henning Bliddal, MD, DMSc, Professor & Head of The Parker Institute,
Bispebjerg and Frederiksberg Hospital.
Nordre Fasanvej 57, DK 2000
Frederiksberg, Denmark
e-mail: henning.bliddal@regionh.dk

Statistical Advisor:

Robin Christensen, BSc, MSc, PhD (Biostatistician); Head of Musculoskeletal Statistics Unit, The Parker Institute.

Statistical Analyst:

Sabrina M. Nielsen, BSc, MSc (Biostatistics fellow); Musculoskeletal Statistics Unit, The Parker Institute.

INTRODUCTION

The knee is the weight-bearing joint most commonly affected with osteoarthritis (OA) and the cardinal symptoms are pain and loss of function. Decreased mobility leading to muscle atrophy, an accelerated decline in physical function, and the inability to engage in activities of daily living such as walking and climbing stairs are clinical consequences of OA and may imply poor quality of life and loss of independence. The risk of incident OA increase with obesity, generalized OA, knee malalignment and synovitis. Inflammation plays an important role in the pathogenesis of pain, the hallmark of (OA).

Musculoskeletal UltraSound (US) is able to detect synovial perfusion and effusion in painful knee OA, which are signs of an inflammatory "flare" in the joint. Osteoarthritis is mainly defined by radiological abnormalities, relying on changes in bone. US is valuable in the early detection of OA as it can define the type and extent of bone and in some degree also cartilage damage and has a potential for monitoring OA progression. Previously we tested evaluated the clinimetrical properties associated with US examinations and found a good reliability and reproducibility of relevant indicators of inflammation.

There is evidence that by treating the obesity of patients with co-occurring OA effectively, the functional status is dramatically improved, with the short-term result equal to that of a joint replacement. Accordingly, the OARSI guidelines recommend that patients with knee OA who are overweight should be encouraged to lose weight and maintain their weight at a lower level. Both obesity and OA are accompanied by low-grade inflammatory changes. It may be speculated that the clinical effect seen after an intensive weight-loss strategy in the obese knee OA patients is associated with a decrease in US indicators of inflammation.

The aim of this study is to evaluate the prognostic value of inflammatory markers detected by US at baseline for weight loss success and clinical response in knee osteoarthritis patients enrolled in an intensive, diet-induced weight loss intervention.

METHODS

Study design and setting

This study includes secondary analyses of the CAROT ('*Influence of weight loss or exercise on CARtilage in Obese knee osteoarthritis patients Trial´*) study. The CAROT study was designed to answer the question of how to maintain the anticipated symptomatic effect while sustaining weight loss for one year in a cohort of well-characterized obese knee OA patients > 50 years (ClinicalTrials.gov identifier: NCT00655941). Participants included in this pragmatic trial were

recruited between November 2007 and August 2008 from the outpatient clinic at the Department of Rheumatology, Frederiksberg Hospital, Frederiksberg, Denmark, through advertisements in newspapers and on the website of the Parker Institute. Additionally, local general practitioners were informed about the possibility of assigning patients to the project.

Participants

Individuals who were >50 years of age with confirmed knee osteoarthritis according to standing radiographs were eligible for inclusion, and obese as defined by a body mass index (BMI) $\geq 30 kg/m^2$. Exclusion criteria were: lack of motivation to lose weight, inability to speak Danish, planned antiobesity surgery, total knee alloplasty and receiving pharmacological therapy for obesity. In total, 192 patients were enrolled in the trial. The participants were asked not to change any medication or nutritional supplement during the study. The study was approved by the ethics committee of the Capital Region of Denmark (H-B-2007 -- 088) and all participants signed an informed consent form.

Intervention

All the participants initially received dietary support for 16 weeks, in order to loose body weight and obtain a clinically important reduction in pain, improvement in physical function and mobility.

The first phase of the study consisted of an 8-week weight reduction program where the participants were using either an all-provided very low energy diet (VLED) with 420 - 554 kcal/d (1743 - 2327 kJ/d) or a low energy diet (LED) with 810 kcal/d (3402 kJ/d) in a supervised dietary program (products provided by the Cambridge Diet, the Cambridge Weight plan, UK). The second phase of the study consisted of 8 weeks' hypo-energetic diet program of ≈ 1200 kcal per day (5040 kJ per day) incorporating two formula diet products daily.

Examinations

As part of the examination program in the CAROT study, the participants had a standardized ultrasound examination of their target knee at baseline and after the 16 weeks intervention [1]. The ultrasound examination was preformed prior to and after intervention (week 16), all examinations were done by investigator BFR, who had received special training in MUS. The US data were exported in a DICOM file for later analysis. The US examination was performed with a General Electric, Logiq9™ (General Electric, Milwaukee, I1, USA), using the M12L linear transducer. The B-mode settings were fixed settings adjusted for musculoskeletal examination, with respect for

adjustments in grey-scale gain, focus and depth. The Doppler sensitivity was optimized for low flow with fixed Doppler settings. The Doppler settings ensured that all color pixels were generated by flow and not random noise. The US examination will be tested for each of five separate domains (1) predominantly morphological changes in the medial compartment, (2) predominantly inflammation in the medial compartment, (3) predominantly morphological changes in the lateral compartment, (4) predominantly inflammation in the lateral compartment, and (5) effusion.

The changes in body weight were measured on a decimal weighing scale (TANITA BW-800, 'Frederiksberg Vægtfabrik', Copenhagen, Denmark). Height was measured using a stadiometer, rounding off the values to the nearest 0.5 cm. Lean body mass (LBM, kg), body fat (kg), bone mineral density (BMD) (g/cm^2) and bone mineral content (BMC) (g) were determined by dual energy X-ray absorptiometry using a Lunar DPX IQ Full Body Bone Densitometer (GE Medical Systems, Madison, WI, USA) and was measured at baseline and after 16 weeks' diet therapy.

Bi-plane weight-bearing semi-flexed (15^0) radiographs were taken of the target knee (in case of bilateral symptoms we used the most symptomatic knee); one in the posteroanterior view and one in the lateral-medial view. They were obtained at baseline, using a Philips Optimus apparatus, and the same radiographers using a standardized protocol carried out all examinations at the same department of radiology.

Variables and outcomes

Our co-primary outcomes are the prognostic value of the US baseline score (predominantly inflammation in the medial compartment) [2], for weight loss success (i.e. loss of more than 10%) and clinical response (i.e. meeting the OMERACT-OARSI responder criterion), after the intervention (week 16) [1,3].

Secondary outcomes will be the similar prognostic value of US baseline scores for 1) predominantly morphological changes in the medial compartment, 2) predominantly morphological changes in the lateral compartment, 3) predominantly inflammation in the lateral compartment, and 4) effusion, as well as the prognostic value of clinical symptoms including the Knee injury and Osteoarthritis Outcome Score (KOOS) subscales, being normalized scores, 100 indicating no symptoms and 0 indicating extreme symptoms.

STATISTICAL METHODS

Population

All analyses will initially be conducted according to the intention-to-treat (ITT) principle, i.e. analyzing participant outcomes according to the group they were randomized.

Statistical analyses

All analyses will be carried out using the statistical software R (version 3.2.3) [4].

Demographic and baseline characteristics

The characteristics of the patients will be described using descriptive statistics presenting continuous outcomes as means with corresponding standard deviations (SD), non-normally distributed continuous variables and ordinal outcomes as medians with corresponding interquartile range (IQR), and binary outcomes as numbers with corresponding percentages.

Primary analyses

Co-primary analyses will consist of simple -, adjusted -, fully adjusted multiple logistic regression analyses for each of the co-primary outcomes, (1a) weight loss success and (1b) meeting OMERACT-OARSI responder criteria at 16 weeks, with the predictor inflammation in the medial compartment. Adjusted multiple logistic regression analyses will be adjusted for gender, age, BMI, and VAS Global at baseline. We will refer to a ”fully adjusted model” (i.e. also multiple logistic regression analyses) which contains the statistically significant results from univariate logistic regression analyses (see variable in Table 1), as we anticipate that many of the independent variables will appear significantly associated with outcome (i.e., either weight loss or clinical response); when we construct multiple logistic regression equations, only some of the independent variables will remain (independently) statistically significantly associated with outcome.

Secondary analyses

Secondary analyses will consist of simple -, adjusted -, fully adjusted multiple logistic regression analyses for each of the co-primary outcomes, weight loss success and meeting OMERACT-OARSI responder criteria at 16 weeks, with the each of the predictors:

- US – Predominantly inflammation in the medial compartment
- US - Predominantly morphological changes in the medial compartment
- US - Predominantly morphological changes in the lateral compartment
- US - Inflammation in the lateral compartment
- US - Effusion

- Kellgren-Lawrence score medial joint compartment
- Kellgren-Lawrence score total
- Weight at baseline
- BMI at baseline
- Gait Biomechanics:
    - 1^{st} peak knee external adduction moment
    - 2^{nd} peak knee external adduction moment
    - Knee external adduction moment impulse
    - 1^{st} knee external flexion moment
- 6min walk
- Knee effusion

No corrections will be applied for multiple testing.

Missing data

In order to adhere to the ITT-principle, missing outcome data will be replaced with the value at baseline, i.e. baseline observation carried forward (BOCF), by assuming no response. If data is missing for the predictors, the patient(s) will be excluded from the analysis.

Other analyses

Pre-specified sensitivity analyses will be conducted for only the primary analyses, using only the population of completers, i.e. complete case (CC) analysis.

RESULTS

Anticipated outline

Table 1.

Demographic and clinical characteristics of the patients at baseline

Variable	Mean ± SD	Min	Max
Female, no. (%)			
Age (years)			
Duration of OA symptoms (years)			
Height (m)			
Weight (kg)			
BMI (kg/m^2)			
Lean body mass (kg)			
Lean body mass (%)			
Fat mass (kg)			
Fat mass (%)			
Bone mineral content (%)			
Current smokers, no. (%)			
Plasma glucose (mmol/L)			
C-reactive protein (mg/dL)			
Kellgren and Lawrence – Grade 1 or 2, no. (%)			
Kellgren-Lawrence score medial joint compartment			
Kellgren-Lawrence score total			
Gait Biomechanics:			
1st peak knee external adduction moment			
2nd peak knee external adduction moment			
Knee external adduction moment impulse			
1st knee external flexion moment			
KOOS pain (0-100)			
KOOS symptoms (0-100)			
KOOS ADL (0-100)			
KOOS sports/recreation (0-100)			
KOOS QOL (0-100)			
VAS pain			
VAS disability			
VAS global			
SF-36 score, PCS			
SF-36 score, MCS			
6min walk (m)			
Knee effusion, n (%)			
Aspiration No.			
Aspiration Volume (mL)			
[1]US - Predominantly inflammation in the medial compartment	Median:		
[1]US - Predominantly morphological changes in the medial compartment	Median:		
[1]US - Predominantly morphological changes in the lateral compartment	Median:		
[1]US - Inflammation in the lateral compartment	Median:		
[1]US - Effusion	Median:		

Abbreviations: ADL, Function in daily living; BMI, body mass index; KOOS, Knee injury and Osteoarthritis Outcome Score; MCS, mental Component summary; OA, osteoarthritis; OR, odds ratio; PCS, physical Component Summary; US, ultra sound, QOL, Quality Of Life; SF-36, short-form 36.

[1]For the subsequent logistical regression analysis these will be included as variables coded as above/below the median.

Table 2.

Univariate logistic regression analyses: Possible predictors for weight loss success or a good clinical outcome after 16 weeks on an intensive dietary intervention

	Weight loss: Simple regression analysis	**OMERACT-OARSI: Simple regression analysis**
Predictor	**Crude OR (95% CI)**	**Crude OR (95% CI)**
Female		
Age (years)*		
Duration of OA symptoms (years)*		
Height (m)*		
Weight (kg)*		
Weight, above median		
BMI (kg/m^2)*		
BMI (kg/m^2), ≥40		
Lean body mass (kg)*		
Lean body mass (%)*		
Fat mass (kg)*		
Fat mass (%)*		
Bone mineral content (%)*		
Current smoker		
Plasma glucose (mmol/L)*		
C-reactive protein (mg/dL)*		
Kellgren and Lawrence – Grade 1 or 2		
Kellgren-Lawrence score medial joint compartment, above median		
Kellgren-Lawrence score total, above median		
Gait Biomechanics:		
1st peak knee external adduction moment, above median		
2nd peak knee external adduction moment, above median		
Knee external adduction moment impulse, above median		
1st knee external flexion moment, above median		
KOOS pain (0-100)*		
KOOS symptoms (0-100)		
KOOS ADL (0-100)*		
KOOS sports/recreation (0-100)*		
KOOS QOL (0-100)*		
VAS pain*		
VAS disability*		
VAS global*		
SF-36 score, PCS*		
SF-36 score, MCS *		
6min walk (m), above median		
Knee effusion		
Aspiration No.*		
Aspiration Volume (mL)*		
US - Inflammation in the medial compartment, above median		
US - Morphological changes in the medial compartment, above median		
US - Morphological changes in the lateral compartment, above median		
US - Inflammation in the lateral compartment, above median		
US – Effusion, above median		

Abbreviations: ADL, Function in daily living; BMI, body mass index; KOOS, Knee injury and Osteoarthritis Outcome Score; MCS, mental Component summary; OA, osteoarthritis; OR, odds ratio; PCS, physical Component Summary; US, ultra sound, QOL, Quality Of Life; SF-36, short-form 36.
*The estimate is a slope.

Table 3.

Multiple logistic regression analyses: Possible predictors for weight loss success or a good clinical outcome after 16 weeks on an intensive dietary intervention

	Weight loss: Multiple logistic regression analyses		**OMERACT-OARSI: Multiple logistic regression analyses**	
Predictor	**OR[1] (95% CI)**	**OR[2] (95% CI)**	**OR[1] (95% CI)**	**OR[2] (95% CI)**
US - Inflammation in the medial compartment, above median				
US - Morphological changes in the medial compartment, above median				
US - Morphological changes in the lateral compartment, above median				
US - Inflammation in the lateral compartment, above median				
US - Effusion, above median				
Kellgren-Lawrence score medial joint compartment, above median				
Kellgren-Lawrence score total, above median				
Weight, above median				
BMI (kg/m^2), ≥40				
Gait Biomechanics - 1st peak knee external adduction moment, above median				
Gait Biomechanics - 2nd peak knee external adduction moment, above median				
Gait Biomechanics - Knee external adduction moment impulse, above median				
Gait Biomechanics - 1st knee external flexion moment, above median				
6min walk, above median				
Knee effusion				

[1]Hypothesis driven (according to protocol: adjusted for gender, age, BMI, and VAS Global at baseline)
[2]Data driven (contains the statistically significant results from corresponding the univariate regression analysis)

REFERENCES

1. Riecke, B. F., Christensen, R., Christensen, P., Leeds, A. R., Boesen, M., Lohmander, L. S., Astrup, A., Bliddal, H. (2010). Comparing two low-energy diets for the treatment of knee osteoarthritis symptoms in obese patients: a pragmatic randomized clinical trial. *Osteoarthritis Cartilage, 18*(6), 746-754. doi: 10.1016/j.joca.2010.02.012
2. Riecke, B. F., Christensen, R., Torp-Pedersen, S., Boesen, M., Gudbergsen, H., & Bliddal, H. (2014). An ultrasound score for knee osteoarthritis: a cross-sectional validation study. *Osteoarthritis Cartilage, 22*(10), 1675-1691. doi: 10.1016/j.joca.2014.06.020
3. Christensen, P., Bliddal, H., Riecke, B. F., Leeds, A. R., Astrup, A., & Christensen, R. (2011). Comparison of a low-energy diet and a very low-energy diet in sedentary obese individuals: a pragmatic randomized controlled trial. *Clin Obes, 1*(1), 31-40. doi: 10.1111/j.1758-8111.2011.00006.x
4. R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.