Statistical Analysis Plan

Ultrasound detected pathology of the entheses in the lower limb in an age and gender stratified sample of healthy participants: a cross-sectional study (v. December 2nd 2016)

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Introduction

Ultrasound (US) examination of the entheses, defined as the tendons, ligaments and capsule insertions into the bone, is increasingly used, in order to document pathological changes in e.g. PsA and SpA. Grey-scale US is used to assess morphological changes and Doppler US to assess increased blood flow. The OMERACT US sub group has made a definition of US feature in enthesopathy including loss of normal architecture, thickened tendon at bone attachment, various bony changes, and Doppler activity (1). In addition, expert groups have worked on definitions on both acute and chronical changes detected by US seen in the area of the entheses in order to identify which anatomical structures and areas should be included in the evaluation (2; 3).

Various scoring systems have been proposed for the evaluation of both acute disease activity and permanent morphological changes (4-7). Most focus has been on the examination of the lower limp and agreement has been reached on the assessment the enthesis at the knee (quadriceps tendon, patella ligament) and at the heel (plantar aponeurosis and Achilles tendon) (3-7).

The features mostly assessed are thickness, architecture and echogenicity of the entheses on grey-scale US and increased flow assessed on Doppler examination. Little is known about the weather similar US abnormalities in the entheses can be find in asymptomatic subjects, which for instance is the case when Doppler activity, assumed to be an indicator of abnormal flow, can be seen in both the Achilles tendon and synovial tissue in the hand in healthy subjects (8; 9). It is relevant to find a cut-off for US enthesis changes in order to optimise the use of US examination for a diagnostic purpose in diseases characterised by pathology in this area.

The aim of this study was to assess the appearance of grey-scale and Doppler abnormalities in the entheses of the lower limb in healthy subjects. This was done by applying two US scoring (GUESS and MASAI) systems for assessment of the entheses of the lower limb in an age-stratified group of subjects with asymptomatic tendons (4; 7). Doppler activity was only registered if it was seen less than 2 mm from the bony attachment as Doppler more than 2 mm from the attachment was defined as free tendon pathology by the expert panel (3; 10). To the best of our knowledge, the appearance of these US features in an age-stratified group of subjects with asymptomatic tendons has not been investigated.

Methods

Participants, recruitment and ethics

The study group was formed by 64 subjects recruited from the Copenhagen General Population Study/The Copenhagen City Heart study (11). Eight women and eight men in each decade from 20 years to 59 years were included. For each decade a random sample of 25 persons were chosen at a time and supplementary samples added in case of shortage of recruits in the decade until the eight women and eight men in each decade were obtained. The procedure was first come first served until the full number of 64 completers had been reached. The volunteers were interviewed by the secretary for inclusion and exclusion criteria, without any involvement of the investigators. Inclusion criteria were: age over 20 years and no history of lasting tendon disease in the lower extremities. Exclusion criteria were: presence or previous signs of tendon disease in the lower extremities, more than eight hours sport activities per week at any time, inflammatory systemic disease, pregnancy, and important medical conditions *e.g.* diabetes mellitus, severe heart, lung or kidney diseases.

The study was approved by the local ethical committee (KF) 01-149/03. In-formed consent was obtained from the participants before inclusion.

Questionnaire
The subjects were asked about basic characteristics, present and previous injuries from tendons, tendon problems in the close family, smoking and alcohol habits, workload (sedentary/ physically active) and sport activities.
Furthermore the Norwegian version of the *Victorian Institute of Sport Assessment* (VISA) scale was filled in by all participants (12). This is an index to assess severity of jumper's knee ranging from 0 to 100, where 100 represent no problems and 0 extreme problems.

Clinical examination
The anterior knee tendons, the Achilles tendon and the fascia plantaris were examined. Pain at palpation, enlargement of the tendons or the peritendinous tissues, and functional tests were included. All the examinations were performed by an experienced rheumatologist (HB).

Blood screening
Blood was analysed for: haemoglobin, plasma creatinine, plasma sodium, plasma potassium, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), plasma alkaline phosphatase, and plasma alanine aminotransferase

Ultrasound
Examinations were performed with a Logiq 9 (GE Medical, Milwaukee, WI, USA) using a 14 MHz centre frequency linear array transducer. The same preset was used for all US examinations. Only the dominant leg was examined (classified according to the dominant arm). Ultrasound examinations were performed by a radiologist experienced in musculoskeletal ultrasound (MJK).

The following five insertion areas were evaluated
1. The quadriceps tendon at the base of the patella.
2. The patellar tendon at the insertion on patella and tuberositas.
3. The patellar tendon at the insertion on tuberositas.
4. Achilles tendon at the insertion on calcaneus
5. Fascia plantaris at the insertion on calcaneus

Positioning of the examined person
The knee tendons were examined with the subjects in supine position and the tendons of the food were examined in the prone position. The grey scale US was performed with the knee in a 20 degree flexed position, obtained with a 10 cm pad under the knee. The Doppler examination was made with the knee fully extended (13). The Achilles tendon and the fascia plantar aponeurosis were examined with the ankle in neutral position with the feet hanging over the edge of the examination table at 90 degrees flexions of the ankle.

Image evaluation
The imaged were evaluated with slight modifications according to two validated scoring systems for US abnormalities in the entheses at the lower limp (GUESS and MASAI). The MASAI score includes the triceps tendon enthesis, while we only scored the tendons of the lower limb. Furthermore, both GUESS and MASAI scores are bilateral, while we only scored the dominant side. To compare the scores, the sum of the two scoring systems were divided with two and in the MASAI score also the grading from the triceps tendon was subtracted (4, 7). Doppler activity had to <2mm from the bone surface in order to be evaluated as part of the entheses as purposed by the expert panel (3). The pathological changes evaluated are listed in Table II. All images were evaluated by two persons in consensus with experience in musculoskeletal US 12 and 3 years, respectively.

Statistical methods
The characteristics of the participants will be described for each gender and decade using descriptive statistics presenting continuous outcomes as means with corresponding standard deviations (SD), ordinal outcomes as medians

```
d=read.table("clipboard",header=TRUE)
d;attach(d);head(d)
Gender<-as.factor(Gender)

#Logistic regression for binary outcome variables - example for Q_tendon_thickness
Q_tendon_thickness<-as.factor(Q_tendon_thickness)
model_LR<-glm(Q_tendon_thickness~Decade+Gender+Decade*Gender, family=binomial)

  anova(model_LR, test="Chisq")

#Proportional odds model for ordinal outcome variables - example for Q_enthesit
Q_enthesit<-as.factor(Q_enthesit)
Q_enthesit<-ordered(Q_enthesit, levels = c("0", "1", "2", "3"))
model_PO1<-vglm(Q_enthesit~Decade+Gender+Decade*Gender,propodds)
summary(model_PO1)
 model_PO2<-vglm(Q_enthesit~Decade+Gender,propodds)
 summary(model_PO2)
pchisq(deviance(model_PO2) - deviance(model_PO1), df = df.residual(model_PO2) -
 df.residual(model_PO1))

#Linear model for continious outcome variables - example for GUESS score
 model_LM<-lm(GUESS~Decade+Gender+Decade*Gender)
  anova(model_LM)
```

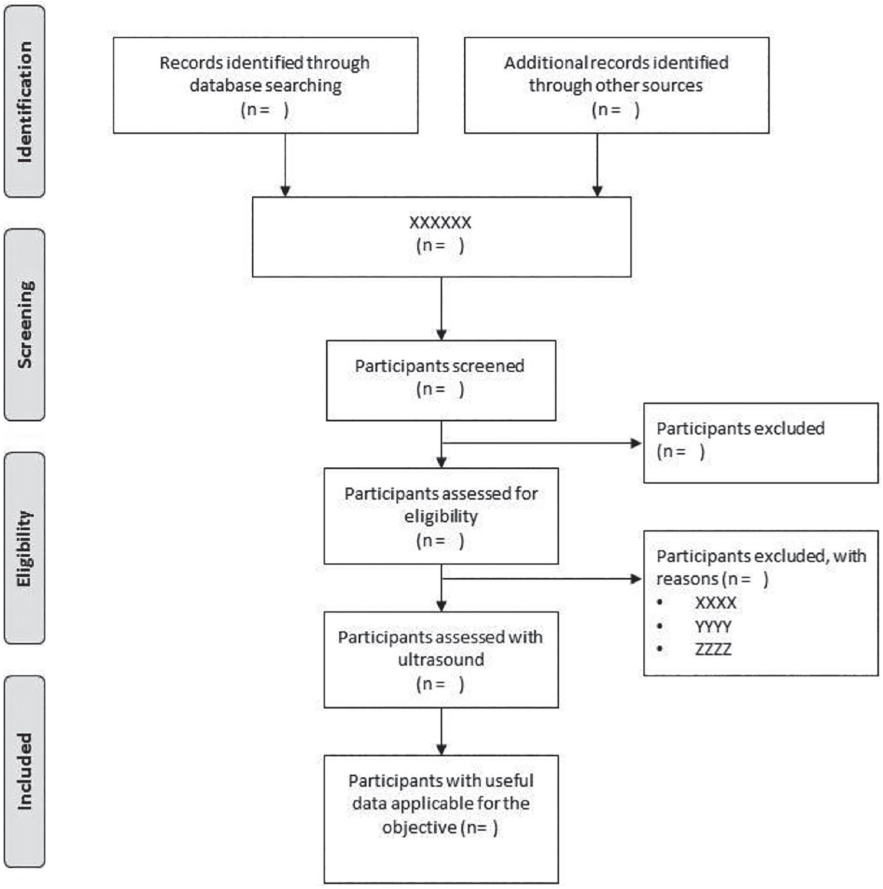

Fig. 1. Flow diagram.

with corresponding interquartile range (IQR), and binary outcomes as numbers with corresponding percentages.

The findings of US pathology will initially be described for each gender and decade using descriptive statistics. Subsequently, data will be analysed with statistical models with the fixed factors, gender (man or woman), age (20-29, 30-39, 40-49, 50-59), and the interaction between these, in order to investigate whether mutual associations exist between these factors and findings of US pathology.

For binary outcome variables logistic regression models will be used, for ordinal outcome variables, such as rating 0–3, proportional odds models will be used, and for continuous variables, such as GUESS and MASEI score, linear models will be used. No correction for multiple testing will be done. All models will be tested for their assumptions. The analyses will be done using the statistical programme R (v. 3.2.3) (14) with the package VGAM (15). The following programme code will be used:

Results

Anticipated outline

Table I. Participant characteristics

Variable	Sex*	Age 20 - 29	Age 30 - 39	Age 40 - 49	Age 50 - 59	Total
Age, mean (SD)	♀ ♂					
BMI (kg/m^2), mean (SD)	♀ ♂					
Smokers, n (%)	♀ ♂					
Alcohol – more than recommended, n (%)*	♀ ♂					
Tendon problems in the closest family, n (%)	♀ ♂					
Former muscle sprain, n (%)	♀ ♂					
Workload – sedentary, n (%)	♀ ♂					
Sports active (0:none 1:moderate 2:intensive),median (IQR)	♀ ♂					
VISA-knee score, mean (SD)	♀ ♂					
Blood screening – abnormal, n (%)	♀ ♂					
Clinical findings – abnormal, n (%)	♀ ♂					

IQR, inter-quartile range; n, numbers; SD, standard deviation *Eight females and eight males in each age subgroup. Total n = 64 **The national board of health recommends not more than 14 units /week for women and 21 for men.

Table II. Findings of US pathology.

Variable	Sex*	Age 20 - 29	Age 30 - 39	Age 40 - 49	Age 50 - 59	Total
Quadriceps Increased tendon thickness, n (%)	♀ ♂					
Quadriceps Changed Tendon structure, n (%)	♀ ♂					
Quadriceps Enthesit (0-3), median (IQR)	♀ ♂					
Quadriceps Doppler (0-3) , median (IQR)	♀ ♂					
Quadriceps Erosion (0-3) , median (IQR)	♀ ♂					
Prox. Patella lig. Increased tendon thickness, n (%)	♀ ♂					
Prox. Patella lig. Changed Tendon structure, n (%)	♀ ♂					
Prox. Patella lig. Enthesit (0-3) , median (IQR)	♀ ♂					
Prox. Patella lig. Doppler (0-3) , median (IQR)	♀ ♂					
Prox. Patella lig. Erosion (0-3) , median (IQR)	♀ ♂					
Dist. Patella lig. Increased tendon thickness, n (%)	♀ ♂					
Dist. Patella lig. Changed Tendon structure, n (%)	♀ ♂					
Dist. Patella lig. Enthesit (0-3) , median (IQR)	♀ ♂					
Dist. Patella lig. Doppler (0-3) , median (IQR)	♀ ♂					
Dist. Patella lig. Erosion (0-3) , median (IQR)	♀ ♂					
Dist. Patella lig. Bursa (±), n (%)	♀ ♂					
Achilles tendon Increased tendon thickness, n (%)	♀ ♂					
Achilles tendon Changed Tendon structure, n (%)	♀ ♂					
Achilles tendon Enthesit (0-3) , median (IQR)	♀ ♂					
Achilles tendon Doppler (0-3) , median (IQR)	♀ ♂					
Achilles tendon Erosion (0-3) , median (IQR)	♀ ♂					
Achilles tendon Bursa (±), n (%)	♀ ♂					
Fascia plantaris Increased tendon thickness, n (%)	♀ ♂					
Fascia plantaris Changed Tendon structure, n (%)	♀ ♂					
Fascia plantaris Enthesit (0-3) , median (IQR)	♀ ♂					
Fascia plantaris Doppler (0-3) , median (IQR)	♀ ♂					
Fascia plantaris Erosion (0-3) , median (IQR)	♀ ♂					
GUESS score (0-18), mean (SD)	♀ ♂					
MASEI score (0-57) , mean (SD)	♀ ♂					

IQR: inter-quartile range; n: numbers; SD: standard deviation. *Eight females and eight males in each age subgroup. Total n=64.

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