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Protocol

Biomarkers in early stage of Spondyloarthritis

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Introduction

Axial spondyloarthritis is a group of inflammatory rheumatic conditions with inflammatory back pain caused by inflammation in the sacroiliac joints (SIJ) and back as hallmark. There are several subgroups of this condition, including psoriatic arthritis, reactive arthritis, enteropathic spondyloarthritis and undifferentiated SpA (1). Depending on the degree of structural damage in the SIJ, axial spondyloarthritis can be divided into non-radiographic axial spondyloarthritis (nr ax-SpA) and radiographic axial spondyloarthritis (ax-SpA) including ankylosing spondylitis (AS) (2).

The diagnoses of SpA and AS currently relies on the Assessment of Spondyloarthritis International Society criteria (ASAS) and the Modified New York criteria (3) including a history of inflammatory back pain, findings at clinical examination and bone marrow oedema (BME) at MRI supported by positive HLA B27 and high C-reactive protein (CRP). By using those criteria especially findings at MRI it seems that patients are diagnosed as having SpA in an early stage, but they do not fulfil the criteria for SpA or AS at a long time, which leaves the consideration that findings at MRI among some of those patients might be caused by overuse instead of an autoimmune-related disease.

Currently, no laboratory test or biomarker is cable of differencing between patients with early-stage SpA and persisting low back pain of other causes. HLA B27 is not an acute biomarker but a prognostic marker for the development of SpA. Approximately 92% of patients with AS is HLA B27 positive (4). Unfortunately, a positive HLA B27 status is detected in up to 10% of healthy individuals (5) and the prevalence of HLA B27 positive individuals in the background populations vary greatly depending on the geographical location (6).

CRP is commonly used as an acute biomarker in different inflammatory conditions. Previous studies have shown that only 40-60% of AS-patients with clinical signs of active disease have elevated levels of CRP (7). Levels of CRP have been correlated with MRI inflammation in AS patients treated with biological agents in the previous study (8).Furthermore, several studies have proven the use of CRP as a marker of future structural damage (9,10). With High Sensitive CRP (hsCRP) it is possible to detect small changes in CRP below 6.0 ml/L (11) and a previous study regarding disease activity of AS-patients and levels of hsCRP suggests that hsCRP is superior to CRP when detecting inflammatory disease activity (10).

Different cytokines like interleukin 17/22/23 (12-17) and antibodies against CD74 (18) have previously been investigated with the purpose of identifying a specific biomarker for SpA and AS. Until now, no specific biomarker for AS has been widely accepted.

Earlier studies regarding the complement C3 split product C3d have shown a correlation between inflammatory diseases like AS and elevation of C3d (19-21). Those studies were based on a small number of cases, but a newer study from 2016 has shown a correlation between different biomarkers including C3 and new fatty lesions at MRI in Golimumab treated AS patients (22). Another study from Wang C. et al. has reported increased levels of C3 in patients with severe AS compared to healthy controls (23).

To be able to discriminate between changes at MRI indicators of autoimmune disease vs overuse specific indicators for inflammation in the early-state is needed as well as more specific evaluation of the findings at MRI.

Objective

The purpose of this study is to investigate the predictive value of baseline levels of C3d and HsCRP to identify bone marrow oedema at MRI of the sacroiliac joints at baseline in early-stage SpA patients.

Method

Design

The study is a prospective cohort study including patients, aged 18-45 years, having unspecific low back pain registered in the Spines of Southern Denmark (SSD) cohort including patients referred to the Spine Centre of Southern Denmark.

At baseline, all patients filled in a standardized questionnaire and were interviewed identifying inflammatory symptoms followed by a clinical examination focusing on symptoms related to the SIJ. Blood samples were taken and analyzed for HLA-B27 (prognostic biomarker). Furthermore, all patients underwent an MRI of the spine, including the SIJ.

In total, 1037 patients were included, 696 had blood tests taken and gave consent for further use for research purposes.

For research purposes, the blood samples were stored in the Molecular Biology of Infectious Agents in the Early Diagnosis of Spondyloarthritis biobank (MICSA). The biobank is hosted by the research group at Graasten Rheumatological Hospital. Of the 696 patients who gave consent,188 patients were examined by a Rheumatologist at baseline. Of those 96 patients fulfilled the criteria of having axSpA according to The Assessment of SpondyloArthritis international Society (ASAS) criteria, 38 patients had inflammatory changes at MRI or had one feature consisting of spondyloarthritis, but did not fulfill the ASAS criteria. 82 randomly selected patients with low back pain were included as a control group.

In total, 216 patients were defined as the cohort included for further analysis in this study.

Data:

The Department of Clinical Biochemistry at Vejle Hospital has previously assisted in the collection and storage of MICSA samples. It is technically possible to use the stored frozen MICSA samples for analyzing C3d.

In total, 184 (of 216) baseline samples were accessible for further analysis. The baseline samples will be analyzed for C3d with the use of EDTA (ethylenediaminetetraacetic acid) plasma and treated according to the local protocol for such analyses.

MRI

All patients in the MICSA cohort have had an MRI of the spine, including the SIJ at baseline. Two experienced radiologists have systematically described the MRI regarding signs of inflammatory changes, including BME.

Statistical analysis plan (SAP)

STATA (version 15.0) will be used for statistical analysis. Parametric data will be reported as the mean and standard derivation (SD) or One-way analysis of variance (ANOVA). In between-group comparisons for continuous and categorical demographic variables will be performed with the independent sample t-test and Pearson Chi-square test or Fisher´s exact test. The Kruskal-Wallis test and interquartile range will be used to describe nonparametric data. Logistic regression analysis will be used to test independent variables

as predictive factors for BME detected by MRI, resulting in odds-ratios, sensitivity, specificity, and area under the curve (AUC). This will be followed by multivariate logistic regression to evaluate the potential effect of other variables. A p-value < 0.05 is considered statistically significant.

Sample size and power calculation:

A previous study with patients suspected of having spondyloarthritis has found a significant difference in mean levels of C3 between groups with 23 and 22 participants, respectively.

By the use of means and SD from the above study and alfa= 0.05, beta= 0.2, power 0.8 and enrollment ratio 1:2 the sample size in our study is calculated to n:10 and n:20 respectively.

It is therefore expected, that a significant difference in C3d levels between the three groups in the present study can be detected based on the size of each group in the MISCA cohort.

Project period

The project period will be from marts 2019 to December 2019. After this, all data will be hosted as part of the MICSA-study.

Economy

This project is part of a PhD-project. The biochemical analysis will be financed by external funding.

Publication

The results will be published in peer-reviewed rheumatological papers.

Ethics

The steering committee for MICSA and SDD will be invited to participate in the study and will be included as authors according to the Vancouver Protocol. Data disclosure agreement will be made before starting the project.

The study is part of a collaborative study between the MICSA and SDD research group and is registered by the Ethical committee *VEK nr. 2011-0029* and S-20140050.

Patients who have withdrawn the consent will be excluded from the population.

Data security

Collected data will be stored and analyzed at a secure Sharepoint profile with logged access. Data will be pseudo-anonymized when information from all relevant sources is collected.

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