Complement C3d is not associated with axial spondyloarthritis and magnetic resonance imaging changes at the sacroiliac joint

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Title: "Complement C3d is not associated with axial spondyloarthritis and MRI changes at the sacroiliac joint."

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Abstract:

Objectives:
To investigate the associations between complement C3d and inflammatory and structural changes by MRI at the sacroiliac joints in a group of patients suggestive of axial spondyloarthritis according to the Assessment of Spondyloarthritis International Society (ASAS) criteria.

Methods:
A cross-sectional study of patients referred to the Spine Centre of Southern Denmark due to unspecified low back pain (Spines of Southern Denmark cohort). The patients were divided into three groups: Group 1: Patients fulfilling the Assessment of SpondyloArthritis International Society (ASAS) criteria for axial spondyloarthritis (axSpA, n=96); Group 2: Patients with either a positive MRI of the SIJ and no spondyloarthritis features, or a negative MRI of the SIJ but positive HLA-B27 and one spondyloarthritis feature (non-axSpA, n=38); Group 3: Patients with unspecified low back pain >3 months (control group n=82). Complement C3d was measured with the double-decker rocket immunoelectrophoresis and evaluated in relation to the group division and baseline findings by SIJ MRI.

Results: One hundred and eighty-four C3d analyses were performed. The mean level of C3d was 33.8 AU/ml with a standard deviation of 8.1. There were no differences between the C3d levels in the three patient groups, the mean values being: axSpA=34.3 AU/ml
(SD=7.9), non-axSpA=33.5 AU/ml (SD=6.9), and control group=33.4 AU/ml (SD=9.2).

The level of C3d was not related to MRI findings.

**Conclusions:**

Complement C3d was not associated with active or structural SIJ changes on MRI in patients suggestive of axial spondyloarthritis.

Trial Registration: Clinical trial.gov number: NCT04095169
Introduction

The term spondyloarthritis (SpA) covers a group of inflammatory conditions characterised by inflammation in the axial skeleton with the possible affection of peripheral joints and extra-articular sites (1).

Although magnetic resonance imaging (MRI) can demonstrate bone marrow oedema (BME) due to inflammation at the sacroiliac joint (SIJ), there is still a significant delay in the diagnosis of patients with SpA (2). Besides, BME at the SIJ can be due to several non-inflammatory conditions (3,4), and differentiation between inflammatory back pain and back pain due to other causes can be difficult as there are no clinical findings specific for early SpA (5). Because effective medical treatments, such as tumour necrosis factor-alpha (TNF-alpha) and interleukin-17 (Il-17) inhibitors, are available, it is important to detect prognostic biomarkers for early-stage SpA (6) to treat the correct patients early and avoid the unnecessary treatment of patients without SpA.

The exact pathogenesis of SpA is unknown, and a humoral immune response has not previously been considered as a primary pathway in the development of SpA (7). However, studies regarding autoantibodies in patients with axSpA such as CD74 (HLA class II invariant chain)(8), and more recently, antibodies for oxidised collagen type II (7) have suggested a humoral component in the SpA development.

The complement cascade reaction is a part of the humoral innate immune response (9) and has several essential functions, including protection against infections, connecting the innate and adaptive immune system, and removal of immune complexes (10,11). The complement system can be activated by three pathways; "classic pathway", "alternative pathway", and via "mannan-binding lectin" (12).
Activation of the complement system results in the conversion of complement factors leading to elevated complement split products (13). The component C3 is a key factor in the system as all three forms of complement activation lead to the cleavage of C3 to C3b via the enzyme C3 convertase. Subsequently, C3b is cleaved into C3c and C3dg, with C3g and C3d being the final fragments (12,14).

Complement activation as part of the pathogenesis of SpA has previously been investigated, and older studies have shown elevated complement components and split products in patients with ankylosing spondylitis (AS) (15-17). These studies were based on a small number of cases, and due to the lack of MRI accessibility, the AS patients were late-stage fulfilling the New York criteria or Modified New York criteria (18) for AS.

A more recent study on AS patients treated with biological agents has shown a negative correlation between high levels of C3 and fatty lesions by MRI (19), while studies regarding TNF-alpha inhibitor treatment have shown reduced complement activation in SpA patients receiving biological treatment (20).

The purpose of this study was to investigate the associations between complement C3d and inflammatory and structural changes by MRI at the SIJ in a group of patients suggestive of axSpA according to the ASAS criteria.
Methods

The study was a cross-sectional study including patients aged 18-45 years, referred to the Spine Centre of Southern Denmark with unspecified back pain in the years 2011-2013 (the Spines of Southern Denmark cohort (SSD)) (21). The Spine Centre of Southern Denmark is a primary care department for patients with primarily degenerative spine disorders (22). At baseline, all patients answered a standardised questionnaire covering inflammatory symptoms followed by a clinical examination focusing on symptoms related to the SIJ and axSpA in general.

An MRI of the SIJ and whole spine was performed on all patients at inclusion. Three radiologists with substantial knowledge and experience in musculoskeletal radiology evaluated the MRI for signs of inflammation and structural lesions compatible with a diagnosis of AS/SpA. All changes were scored semi-quantitatively using a Danish MRI grading system (23), including bone marrow oedema (BME) as well as erosions, subchondral sclerosis, fatty marrow deposition (FMD) and ankylosis, and the depth of BME and FMD (± >1 cm beneath the joint surface) in addition to the signal intensity of BME (less than spinal fluid or equal to spinal fluid). In the current study, the SIJ BME findings were subsequently classified according to the criteria defined by the Assessment of SpondyloArthritis International Society (ASAS) in 2009 (24) as ASAS MRI-positive and ASAS MRI-negative depending on the amount of BME. ASAS MRI-positive had at least two BME areas on one MRI slice or a BME extending over more than one slice. The different structural changes were scored by the prevalence of their occurrence in the different groups.
The SSD cohort and MRI protocol are described in detail by Arnbak et al. (21). In total, 1037 patients with persisting low back pain >3 months were included in the study. A subsequent study on the SSD cohort (25) encompassing biochemical examination (Human Leukocyte Antigen B27 (HLA-B27) and high-sensitive C-reactive protein (hs-CRP)) as well as a questionnaire and clinical examination was performed based on 1020 patients, 696 of whom gave consent for the further use of blood samples for research purposes (Figure 1).

Overall, 189 of the 696 patients were referred for clinical examination by three rheumatologists due to either:

a. The fulfilment of ASAS criteria for axSpA
b. ASAS MRI-positive SIJ without additional ASAS features
c. HLA-B27 positivity and one further ASAS feature

The remaining 507 patients were eligible as controls (Figure 1).

Based on the rheumatological examination, the patients were divided into three groups based on the clinical and biochemical findings, and a summary statement regarding whether or not the patient was ASAS MRI-positive or MRI-negative: Group 1: Patients fulfilling the imaging or clinical arm of ASAS for axSpA (axSpA n=96). Nine patients fulfilled the ASAS axSpA classification according to the clinical arm only (HLA-B27 positive and at least 2 SpA features); Group 2: Patients with either positive SIJ MRI and no SpA features, or negative SIJ MRI, but positive HLA-B27 and one SpA feature (non-axSpA n=38); and Group 3: Patients with unspecified low back pain >3 months and no inflammatory changes at SIJ MRI (control group n=82) (figure 1).
Patients in the control group were contacted by phone (OH). Among the 507 eligible patients, inclusion was performed continuously until 76 patients had agreed to blood sampling (26). Six of the individuals initially referred to the rheumatologist did not meet the inclusion criteria for the axSpA and non-axSpA groups after rheumatological examination and were transferred to the control group (Figure 1).

After the rheumatological examination and allocation into the three groups, radiography of the SIJ was performed and evaluated according to the modified New York criteria for the patients in the axSpA and non-axSpA group, but not for the control group.

In total, 216 patients were included in the present study.

Biochemical assessment:

A blood sample was obtained at baseline at the Spine Centre of Southern Denmark. Two ml serum was analysed for HLA-B27 and hs-CRP at The Danish Hospital for Rheumatology. The remaining serum after HLA-B27 and hs-CRP testing (1 ml) was stored in the Molecular Biology of Infectious Agents in the Early Diagnosis of Spondyloarthitis (MISCA) biobank. At the time of clinical examination, a second set of blood samples was taken from 189 patients who participated in the clinical examination, as well as the 76 controls. Sera used in the present study were derived from blood samples from the second visits. The mean time from the MRI SIJ to the blood sampling for the C3d analyses was 129 days.

The biobank is hosted by the research group at the Danish Hospital for Rheumatology, Sonderborg. The MISCA cohort is described in detail by Hermansen et al. (26). The stored MISCA samples were transferred from the Danish Hospital for Rheumatology, Sonderborg to the Department of Clinical Biochemistry at Vejle Hospital.
according to local instructions, and the durability and temperature requirements of the samples before analysis were respected.

With the use of 1 ml ethylenediaminetetra-acetic acid (EDTA) plasma and polyclonal rabbit anti-human C3d complement (DakoCytomation Denmark A/S), the quantitation of C3d in the baseline samples was measured by double-decker rocket immunoelectrophoresis (27). The procedure for C3d analysis takes two days: Day 1: the preparation of Gel 1 (11.1 ml 1.8% agarose, 11.1 ml 4% PEG, 65 µL anti-C3d), and Gel 2 (8.9 mL 0.9% agarose, 500 µL anti-C3c) followed by electrophoresis with DC 100 V and 15-25 mA.

Day 2: Washing Gels 1/2 for 15 minutes with 0.1 M NaCl, with the subsequent drying and height measurement in millimetres of the C3d-rockets manually by a medical laboratory technologist with many years of experience in C3d analysis. The procedure is described in detail elsewhere (13). The normal range of C3d in a healthy reference population has been measured as 20-52 AU/L. The analytical coefficient of variation (CV) is 9% at the upper normal limit (13).

Figure 1: Patient- and blood sample flow from SSD- and MICSA cohort to the present study with measured C3d samples in each MICSA group
**Statistical analysis:**

Parametric data were reported as the mean and standard derivation (SD). One-way analysis of variance (ANOVA) was used to test differences in mean C3d levels between the MICS A groups (axSpA, non-axSpA, control group). Between-group comparisons for continuous and categorical demographic variables were performed with the independent sample t-test and Pearson Chi-square test. The Kruskal-Wallis test and interquartile range were used to describe nonparametric data.

Logistic regression analysis was used to test the hypothesis of an association between C3d levels and MRI changes, assessing odds-ratios, sensitivity, specificity, and area under the curve (AUC). This was followed by multivariate logistic regression in order to evaluate the potential effect of other variables, such as demographic factors and other biomarkers (HLA B27, Hs-CRP).

A *p*-value <0.05 was considered statistically significant.

STATA (version 15.0) was used for statistical analysis.
Results

Of the 188 blood tests eligible for examination (axSpA n=88, non-axSpA n=35 and control n=65), 184 were analysed correctly.

Four samples had to been excluded due to haemolysis or technical challenges within the analytical process (Figure 1).

In total, the mean level of C3d was 33.8 AU/ml with a standard deviation (SD) of 8.1.

Four patients had C3d levels below normal <20 AU/ml (AxSpA n=3, control group n=1), and two patients had levels above the normal range >52 AU/ml (55 AU/ml and 67 AU/ml, respectively), both belonging to the control group.

There was no statistically significant difference in C3d levels between the three MICSA groups (p-value 0.15) with mean C3d level axSpA=34.3 AU/ml (SD=7.9), non-axSpA=33.5 AU/ml (SD=6.9), and control group=33.4 AU/ml (SD=9.2).

There were significant differences in the prevalence of HLA-B27 and levels of hs-CRP between the axSpA and non-axSpA group (p-values of 0.05 and 0.007, respectively) (Table 1). The prevalence of deep BME lesions and erosions at SIJ was significantly higher in the axSpA group compared to non-axSpA (p-value 0.02/0.02).

No differences regarding biomarkers (HLA-B27 and hs-CRP) between the non-axSpA group and control group were found. There was a significantly higher prevalence of BME (p-value <0.01), deep BME (p-value 0.02), FMD (p-value 0.02) and erosions (p-value <0.01) in the non-axSpA group compared to the control group.
Table 1. Demographics, MRI findings, and blood tests characteristics of the axSpA, non-axSpA and control groups.

<table>
<thead>
<tr>
<th></th>
<th>AxSpA (n=88)</th>
<th>Non-axSpA (n=34)</th>
<th>p-value † (axSpA/non-axSpA)</th>
<th>Controls (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years (SD)</strong></td>
<td>30.8 (5.4)</td>
<td>32.2 (5.9)</td>
<td>0.22</td>
<td>30.6 (5.9)</td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (36.4)</td>
<td>14 (41.2)</td>
<td>0.62</td>
<td>37 (59.7)</td>
</tr>
<tr>
<td>Female</td>
<td>56 (63.6)</td>
<td>20 (58.8)</td>
<td></td>
<td>25 (40.3)</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA B27 positive, n (%)</td>
<td>32 (36.4)</td>
<td>6 (17.7)</td>
<td>0.05 ‡</td>
<td>5 (8.1)</td>
</tr>
<tr>
<td>Hs-CRP, median (IQR)</td>
<td>1.9 (0.6-6.6)</td>
<td>0.4 (0.2-2)</td>
<td>0.007 ‡</td>
<td>0.6 (0.2-1.6)</td>
</tr>
<tr>
<td><strong>Inflammatory changes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BME/ASAS MR-positive, n (%)</td>
<td>82 (85.4)</td>
<td>30 (78.9)</td>
<td>0.34 †</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Depth BME, n (%)</td>
<td>27 (28.1)</td>
<td>4 (10.5)</td>
<td>0.02</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Intensity BME, n (%)</td>
<td>11 (11.5)</td>
<td>1 (2.6)</td>
<td>0.16</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Structural MRI changes:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosions, n (%)</td>
<td>31 (32.3)</td>
<td>5 (13.2)</td>
<td>0.02</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Fatty marrow deposition, n (%)</td>
<td>37 (38.5)</td>
<td>11 (28.9)</td>
<td>0.21</td>
<td>5 (6.1)</td>
</tr>
<tr>
<td>Depth FMD, n (%)</td>
<td>21 (21.8)</td>
<td>3 (7.9)</td>
<td>0.08</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Subcondral sclerosis, n (%)</td>
<td>27 (28.1)</td>
<td>6 (15.7)</td>
<td>0.47</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Ankylosis, n (%)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>n/a</td>
<td>1 (1.21)</td>
</tr>
</tbody>
</table>

*student t-test, †Pearson’s chi² test, ‡Mann-Whitney U test
†P-value comparison between axSpA and non-axSpA group
BME=Bone marrow oedema; FMD=Fatty marrow deposition
Association between C3d levels and MRI findings

There was no statistically significant difference in mean C3d levels between the ASAS MRI-positive and ASAS-negative groups (Student t-test p-value 0.8).

The C3d levels were tested, and the area under the curve (AUC) and cut-off values were determined. The AUC was 0.537 (95% CI: 0.45-0.62), and the best C3d level was 31.2 AU/l with a sensitivity of 62.6% and specificity of 45.5%.

The multivariable logistic regression on demographics (age, sex) and biomarkers (HLA-B27, hsCRP) was tested (Table 2). There was a higher probability of having an ASAS-positive MRI for females (odds ratio (OR): 1.94, p-value: 0.03). Age and C3d did not contribute to higher AUC for ASAS-positive SIJ MRI. By using all co-variables, the AUC increased to 0.63 (Figure 2). The sensitivity and specificity of the multivariable logistic regression model were 75.7% and 46.8%, respectively. The positive predictive value and negative predictive value were 0.66 and 0.58, with an accuracy of 63.6% correctly classified.

Table 2: Associations between demographic variables and biomarkers with the fulfilment of the ASAS definition for positive SIJ MRI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio</th>
<th>Std. Err.</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.03</td>
<td>0.97-1.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>1.94</td>
<td>0.60</td>
<td>1.07-3.55</td>
<td>0.03</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HsCRP</td>
<td>1.06</td>
<td>0.04</td>
<td>0.99-1.14</td>
<td>0.07</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>0.74</td>
<td>0.27</td>
<td>0.36-1.51</td>
<td>0.40</td>
</tr>
<tr>
<td>C3d</td>
<td>0.99</td>
<td>0.02</td>
<td>0.95-1.03</td>
<td>0.70</td>
</tr>
</tbody>
</table>
There was no statistically significant difference in mean C3d levels in relation to the individual MRI findings (BME (p-value: 0.85), BME-depth >1 cm beneath the joint surface (p-value 0.10), erosions (p-value: 0.50), FMD (p-value: 0.13)) tested on the entire population. Furthermore, there was no difference when tested separately for males and females.

There was a significantly higher proportion of females than males with BME changes (females: 57.1%, males: 46.9%, p-value 0.03).

**Radiographical assessment:**

For 104 patients, SIJ radiographs were available, and the classification of the SIJ according to the modified New York criteria (mNYC) (15) was possible. In total, 16 patients fulfilled the mNYC criteria (ax-SpA: n=14, non-axSpA: n=2).

A corresponding C3d measurement was available for 96 patients. The mNYC was fulfilled in 14 patients (14.6%): 13 (13.5%) cases in the axSpA group and 1 (1.0%) case in the non-axSpA group.

Among the axSpA patients, there was no difference in C3d levels between patients fulfilling the mNYC and patients without structural changes at SIJ radiographs (p-value 0.39).

All patients fulfilling the modified New York criteria fulfilled the MRI criteria for sacroiliitis concomitantly.
Discussion:

To the best of our knowledge, this is the first study to investigate the associations of complement C3d and inflammatory and structural changes by SIJ MRI in a population of patients suggestive of axSpA. However, there were no statistically significant differences in C3d levels between the three MICSA groups despite significantly higher frequencies of MRI changes in the axSpA group compared to the remainder. Only two cases had C3d levels above the normal range, both belonging to the control group. Previous data (15-17), including recent studies (20), have reported activation of the complement system in patients with AS compared to the background population. However, in the older studies, patients were classified as having AS due to the New York criteria from 1968 (28) based on structural changes at the SIJ, which most often reflects longstanding disease. Patients in recent studies were eligible for treatment with TNFα-inhibitors, which could also indicate that the patients were not in the early-stage of SpA/AS. In the present study, patients had unspecified low back pain for at least three months before inclusion in the SSD cohort (25), but the individual duration of symptoms is unknown. Furthermore, baseline radiographic SIJ changes in the axSpA and non-axSpA group were evaluated, and there was no difference in C3d levels between the groups.

We investigated the potential association of baseline C3d levels and MRI changes at the SIJ, first for the ASAS positive/negative MRI score, and second for all inflammatory and structural changes, as well as single MRI findings with no significant detectable differences. The interpretation of changes at the SIJ MRI was performed by three experienced radiologists, and their inter-/intra-observer agreement was evaluated in a
previous study (29). We, therefore, believe that the classification of SIJ MRI is highly reliable and does not account for any skewness. Deep BME lesions and erosions were overrepresented in the axSpA group, which is in line with previous studies regarding MRI findings in SpA patients (30). In light of the above, we conclude that baseline levels of C3d were not associated with inflammatory or structural MRI changes at the SIJ.

The quantification and measurement of C3d levels in this study were performed with the use of double-decker rocket immunoelectrophoresis (13). This method has been used for many years in Hospital Lillebaelt, and we believe that it is highly reliable and does not account for significant skewness within the C3d levels. However, the method requires manual measurements in millimetres of the C3d rockets, which is a time-consuming and expensive procedure. Besides a few patients, nearly all of the C3d tests were within the normal range in both the axSpA and control group. This indicates that the complement system is not activated in early-stage SpA.

Other methods, including the time-resolved immuno fluorometric assay (TRIFMA), have been used for the quantification of C3 and activation fragments in other studies (31). We do not believe that using other methods of C3d testing would have significantly changed the results, which is substantiated by a newer study testing C3 levels in 120 axSpA patients without significant changes compared to a control group with the use of the TRIFMA method (32).
**Strengths and limitations:**

This study has some limitations. First of all, patients with apparent signs of inflammatory activity such as severe inflammatory back pain and/or peripheral manifestations are usually initially referred to a department of rheumatology rather than to an outpatient clinic primarily managing back pain conditions of degenerative origin. This could result in selection bias and may influence the lack of differences in C3d results between the groups.

The exact duration of the patients' symptoms is unknown, and it is uncertain at which stage of the disease the baseline SIJ MRI was performed. Secondly, only C3d at baseline was measured. It is possible that C3d levels would increase along with some progression of the symptoms of AS/SpA within six and twelve months of baseline.

In the present study there was an unusually low proportion of HLA-B27 positive (axSpA: 36.4%, non-axSpA: 17.7%), and a higher proportion of females in both the axSpA and non-axSpA group (axSpA: 63.6%, non-axSpA: 58.8%). Furthermore, there was a higher proportion of BME changes at the MRI SIJ in women. This, in contrast to usual observations in the prevalence of HLA-B27 and gender distribution in cohorts of spondyloarthritis patients (33).

We consider that the inclusion of patients from a primary care department for patients with primarily degenerative spine disorders may have contributed to the heterogeneity of the cohort and that biomechanical stress at the SIJ in women with longstanding low back pain and/or postpartum changes at MRI SIJ can have contributed to the higher frequency of BME detected at the MRI SIJ in women. The above result may limit the generalizability of this study to axSpA identified in usual clinical practice.
The mean time from baseline MRI SIJ to the blood sampling for the C3d analyses was 129 days. The relatively long time period between MRI and C3d testing is a limitation when evaluating the association between C3d and inflammatory changes at MRI. However, older studies have demonstrated little variation in C3d levels in patients with rheumatoid arthritis over a three months period (34). Complement activation is a recognised sign of disease flare-up in other rheumatological diseases such as SLE, and we hypothesise that if complement activation was associated with active inflammation in axSpA patients, elevated levels of C3d would be expected in at least a subgroup of patients in the axSpA group as a sign of sustained inflammation.

A strength of this study is the high number of included patients and collected baseline C3d samples. Combined with baseline SIJ MRI and baseline radiography, it was possible to test the association of C3d and MRI changes sufficiently in a large cohort of patients with clinical and radiological suspicion of early-stage SpA. However, the study population still may be too small for subgroup analyses such as radiographic axSpA since the modified New York criteria were fulfilled in fourteen cases only.

In conclusion, Complement C3d was not associated with active or structural SIJ changes on MRI in patients suggestive of axial spondyloarthritis.
Ethics and consent:
The Region of Southern Denmark was the data controller for this project, and it is included in their records of personal data processing activities (file no. 15/38665). The Regional Committee on Human Research Ethics approved the study (S-20140050/S-20190004), and the study was conducted according to the Declaration of Helsinki. The data processing was conducted according to EU and Danish legislation on the processing of sensitive personal information, as well as internal regulations from the Region of Southern Denmark. Analyses were run on pseudonymised data, and the results presented in this manuscript do not enable the identification of single data subjects.

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Conflicts of interest:
The authors declare no conflicts of interest.

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30. Bennett AN, McGonagle D, O'Connor P, Hensor EM, Sivera F, Coates LC et al. Severity of baseline magnetic resonance imaging-evident sacroiliitis and HLA-


Figure 1: Patient- and blood sample flow from SSD- and MICSA cohort to the present study with measured C3d samples in each MICSA group

- **Spines of Southern Denmark (SSD)**
  - (n=1037)
- **Excluded from MICSA (n=341)**
  - n=224, LBP < 3 months
  - n=11, no blood sample present
  - n=106, did not wish to participate/did not sign consent form
- **MICSA cohort**
  - (n=696)
- **Not sufficient amount of serum in biobank for further analysis (n=28)**

- **Referred to rheumatologist (n=189)**
  - Excluded (n=49)
    - n=27, did not wish to participate
    - n=7, missed the consultation
    - n=4, did not sign consent form
    - n=11, other reasons
- **Eligible as controls (n=507)**
  - n=6a
  - n=76b

- **AxSpA (n=96)**
  - n=8
- **Non-axSpA (n=38)**
  - n=3
- **Controls (n=82)**
  - n=17

- **AxSpA C3d (n=88)**
- **Non-axSpA C3d (n=34)**
- **Controls C3d (n=62)**

- **AxSpA (n=88)**
- **Non-axSpA (n=35)**
- **Controls (n=65)**

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*Patients not fulfilling criteria for SpA or being at risk of SpA

*R Random sample from the control group invited to the MICSA project included by author OH.

*Analyses missed during the processing of C3d samples
Table 1. Demographics, MRI findings, and blood tests characteristics of the axSpA, non-axSpA and control groups.

<table>
<thead>
<tr>
<th></th>
<th>AxSpA (n=88)</th>
<th>Non-axSpA (n=34)</th>
<th>p-value(^a) (axSpA/non-axSpA)</th>
<th>Controls (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>30.8 (5.4)</td>
<td>32.2 (5.9)</td>
<td>0.22(^a)</td>
<td>30.6 (5.9)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (36.4)</td>
<td>14 (41.2)</td>
<td>0.62(^b)</td>
<td>37 (59.7)</td>
</tr>
<tr>
<td>Female</td>
<td>56 (63.6)</td>
<td>20 (58.8)</td>
<td></td>
<td>25 (40.3)</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA B27 positive, n (%)</td>
<td>32 (36.4)</td>
<td>6 (17.7)</td>
<td>0.05(^b)</td>
<td>5 (8.1)</td>
</tr>
<tr>
<td>Hs-CRP, median (IQR)</td>
<td>1.9 (0.6-6.6)</td>
<td>0.4 (0.2-2)</td>
<td>0.007(^c)</td>
<td>0.6 (0.2-1.6)</td>
</tr>
<tr>
<td>Inflammatory changes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BME/ASAS MR-positive, n (%)</td>
<td>82 (85.4)</td>
<td>30 (78.9)</td>
<td>0.34(^a)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Depth BME, n (%)</td>
<td>27 (28.1)</td>
<td>4 (10.5)</td>
<td>0.02</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Intensity BME, n (%)</td>
<td>11 (11.5)</td>
<td>1 (2.6)</td>
<td>0.16</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Structural MRI changes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosions, n (%)</td>
<td>31 (32.3)</td>
<td>5 (13.2)</td>
<td>0.02</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Fatty marrow deposition, n (%)</td>
<td>37 (38.5)</td>
<td>11 (28.9)</td>
<td>0.21</td>
<td>5 (6.1)</td>
</tr>
<tr>
<td>Depth FMD, n (%)</td>
<td>21 (21.8)</td>
<td>3 (7.9)</td>
<td>0.08</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Subcondral sclerosis, n (%)</td>
<td>27 (28.1)</td>
<td>6 (15.7)</td>
<td>0.47</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Ankylosis, n (%)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>n/a</td>
<td>1 (1.21)</td>
</tr>
</tbody>
</table>

\(^a\)student t-test, \(^b\)Pearsons chi2 test, \(^c\)Mann-Whitney U test

\(^1\)P-value comparison between axSpA and non-axSpA group

BME=Bone marrow oedema; FMD=Fatty marrow deposition
Table 2: Associations between demographic variables and biomarkers with the fulfilment of the ASAS definition for positive SIJ MRI

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio</th>
<th>Std. Err.</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.03</td>
<td>0.97-1.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>1.94</td>
<td>0.60</td>
<td>1.07-3.55</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Biomarkers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HsCRP</td>
<td>1.06</td>
<td>0.04</td>
<td>0.99-1.14</td>
<td>0.07</td>
</tr>
<tr>
<td>HLA-B27</td>
<td>0.74</td>
<td>0.27</td>
<td>0.36-1.51</td>
<td>0.40</td>
</tr>
<tr>
<td>C3d</td>
<td>0.99</td>
<td>0.02</td>
<td>0.95-1.03</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Figure 2: Sensitivity and specificity of multivariable logistic regression on demographics (age, sex) and biomarkers (C3d, HLA-B27, CPR) in relation to ASAS MRI-positivity.

Area under ROC curve = 0.6333