Chapter 7

Changes in synovial inflammation over time in rheumatoid arthritis

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Abstract

Objectives
To investigate histological changes in synovial inflammation over time in rheumatoid arthritis (RA) patients in the context of anti-cyclic citrullinated peptides (CCP) antibody status.

Methods
Sequential arthroscopic synovial tissue samples were taken from inflamed knee joints of 30 RA patients (18 CCP-positive) and examined for histological features along with immunohistological expression of MMP-13.

Results
The mean time between biopsies was 3.8 (SD 3.2) years. Pathological features of synovial infiltrate did not change in the patients when taken as one group except for an increase in fibrin from 1.03 to 1.80 (p=0.012). Anti-CCP negative patients showed an increase in synovial lining layer thickness from 1.98 to 3.08 (p=0.04) and fibrosis score from 0.50 to 1.75 (p=0.03). Anti-CCP positive patients showed an increase in fibrin score from 0.72 to 1.78 (p=0.008).

There was a higher expression of MMP-13 in anti-CCP positive patients compared to anti-CCP negative patients (2.75 (SD 0.72) vs. 2.00 (0.96), p=0.04). MMP-13 expression correlated well with radiological deterioration over time.

Conclusions
Synovial inflammation persisted despite DMARD treatment, but with different features and radiological outcome in anti-CCP negative and anti-CCP positive RA.
Introduction
Over the years synovial tissue studies in RA have provided insight into local disease processes and changes in inflammation during therapy (1;2) but also during placebo treatment (3). Invariably, these studies have focused on short term changes over weeks or months. The current point of view is that the synovial inflammation, at least on a cellular level, remains constant in spite of therapy (4). However, these studies often used synovial tissues obtained at joint replacement surgery which was shown to be different from RA joints not requiring surgery (5). Also, comparisons between early and late RA were made between separate groups of patients and not by means of sequential biopsies within a cohort. Therefore little is known about changes in synovial tissue infiltration over the years in RA patients.
In a previous study we found a difference in synovial infiltration between anti-cyclic citrullinated peptides (CCP) positive and anti-CCP negative RA patients, even in early stages of RA, with respect to the number of infiltrating lymphocytes (6). Stronger lymphocytic infiltration was present in the synovial tissue of anti-CCP positive patients, which was associated with increased joint destruction.
The present study was set up to extend these findings and investigate whether synovial inflammation change over time and to explore further differences between anti-CCP positive and anti-CCP negative RA.

Patients and methods
We examined synovial tissue specimens obtained from patients with an established diagnosis of RA according to ACR-criteria (7) who underwent two sequential diagnostic or therapeutic arthroscopies of an inflamed knee between 1998 and 2005. The patient group was nearly identical to the one described in our previous report (6). All patients were treated with disease modifying anti rheumatic drugs (DMARDs). Patient sera were tested for the presence of anti-CCP antibodies at the time of the second arthroscopy using the anti-CCP2 antibody ELISA (Immunoscan RA Mark 2; Euro-diagnostica, Arnhem, The Netherlands) according to the manufacturer's instructions with a cut-off value of 25 units. Standard anteroposterior (AP) knee radiographs were taken in all patients and included in the analysis if taken within three months before or after the synovial tissue sampling. Radiographs were scored for severity of osteoarthritis on the Kellgren and Lawrence (K/L) scale (8) for joint space narrowing, osteophytes and sclerosis. This score was divided into two categories depending on the absence (K/L 0-1) or presence of osteoarthritis (K/L 2-4).
Synovial tissue analysis

Synovial biopsies were obtained by arthroscopy in all patients in a standardized manner (9) and analyzed histologically and immunohistochemically.

Histological analysis

Synovial specimens were embedded in paraffin, sectioned at 3 µm and stained with haematoxylin and eosin. Coded sections were scored independently by two observers (MO and IB) for the following histological features: mean synovial lining layer thickness at six random places; mean number of infiltrating lymphocytes, plasma cells and neutrophils from three random high power fields (HPF, 400x); vascularity expressed as the number of blood vessels with detectable endothelial cells in three random HPFs (200x); presence of fibrosis and fibrin (0-4, 0 = absent, 4 = abundantly present). Differences between the observers were resolved by mutual agreement.

Immunohistochemistry

Synovial tissue was collected, processed and scored semiquantitatively as described before (10). Tissue samples were stained for matrix metallo proteinase-13 (MMP 13) (R&D Systems, UK)

Statistical analysis

Statistical analyses were performed using Wilcoxon signed rank test, Chi-square test and non-parametric correlation (Spearman’s rho) where appropriate. All statistical analyses were done with SPSS 11.5 (SPSS Inc, Chicago, USA). P-values <0.05 were considered statistically significant.

Results

A total of 30 RA patients were studied, of whom 18 were anti-CCP positive. Mean (SD) anti CCP-titers were 894 (1004) and 20 (0.6) for anti-CCP positive and anti-CCP negative respectively. All anti-CCP positive patients were females, whereas the anti-CCP negative group contained 5 female and 7 male patients. There were no differences between anti-CCP positive and anti-CCP negative patients concerning age (54.7 ± 12.2 yrs resp. 54.8 ± 12.8, p=0.98), disease duration (11.9 yrs ± 5.9 resp. 9.2 ± 8.2, p=0.12) and number of previous corticosteroid injections of the knee (1.1 ± 1.7 resp. 2.2 ± 4.6, p=0.18). There was a difference in rheumatoid factor positivity (16/18 in anti-CCP positive compared to 4/12 in anti-CCP negative patients, p<0.01) and previous DMARDs (4.8± 1.8 resp. 3.3 ± 2.7, p=0.04) The time
Temporal changes in synovial inflammation

between both arthroscopies was 3.8 (SD 3.2) years for all patients with no difference between anti-CCP positive and –negative patients (p=0.30).

In between biopsies patients were treated with Disease Modifying Anti Rheumatic Drugs (DMARDs). The number of patients using MTX on both timepoints was 14 resp. 11 for anti-CCP positive resp. anti-CCP negative patients at the first biopsy (p=0.32) and 16 resp. 11 at the second biopsy (p=0.36). In between biopsies 16 anti-CCP positive and 11 anti-CCP negative patients changed DMARD therapy (p=0.58). Four patients (two in each group) received TNF-alpha blocking agents in the time between both biopsies, but all four patients discontinued treatment because of lack of response. Table 1 displays the histological scores and K/L scores at both time points. The overall synovial infiltrate did not change in the patients when taken as one group except for an increase in fibrin (p=0.012). Kellgren and Lawrence scores increased over time in all patients (p=0.01). The increase in K/L was also significant in anti-CCP positive patients (p=0.02). Five patients (all anti-CCP positive) progressed from K/L 0-1 to K/L 2-4 (p=0.10).

In anti-CCP negative patients increases were seen in synovial lining layer thickness (p=0.04) and fibrosis score (p=0.03). In anti-CCP positive patients an increase in fibrin score was observed (p=0.008).

At the time of the first biopsy anti-CCP positive patients showed more infiltrating lymphocytes compared to anti-CCP negative patients (72.4 (41.9) vs. 27.3 (22.8), p=0.007).

At the second biopsy differences were observed between anti-CCP positive and anti-CCP negative patients for infiltrating lymphocytes (p=0.008) and synovial lining layer thickness (p=0.03). The change over time in synovial lining layer thickness and fibrosis was significantly different between anti-CCP positive and –negative patients (p-value 0.03 and 0.04 resp.). There was a higher expression of MMP-13 in anti-CCP positive patients compared to anti-CCP negative patients (2.75 (0.72) vs. 2.00 (0.96), p=0.04). Figure 1 shows representative images of MMP-13 expression in anti-CCP positive and anti-CCP negative RA. MMP-13 correlated well with K/L score (rho = 0.675, p=0.03).

Discussion

This study aimed to investigate the changes over time of synovial inflammation in patients with rheumatoid arthritis (RA). With almost four years between synovial biopsies and despite treatment with DMARDs the overall cellular infiltrate did not change. Between both time points there was an increase in fibrin deposition, almost exclusively in anti-CCP positive
patients. In contrast, an increase in synovial lining layer thickness and fibrosis was observed in anti-CCP negative patients.

During that time (secondary) osteoarthritis, displayed by K/L score, increased moderately, predominantly in the anti-CCP positive patients.

Fibrin is a hallmark of longstanding synovial inflammation in RA but generally considered an inactive residue of previous inflammation. Several studies (11;12), however, have shown a pro-inflammatory effect of fibrin on synovitis. Recently, antibodies directed towards citrullinated fibrin were shown to activate macrophages and increase TNFα production (13).

The increase in synovial lining layer thickness and fibrosis in anti-CCP negative patients is remarkable. Like in other chronic inflammatory diseases, in RA, periods with distinct synovial inflammation are alternated with periods of less inflammation. Inflammation may lead to scarring or repair of the synovial tissue which is microscopically recognized as fibrosis, especially in longstanding RA. One study found that fibroblast like synoviocytes, which are the main cell type in hypertrophic synovial lining, are transformed into myofibroblasts by epithelial mesenchymal transition (EMT) and produce increased amounts of extra cellular matrix or fibrosis (14). Perhaps in anti-CCP negative RA the formation of an increased lining layer leads to increased formation of extra cellular matrix and fibrosis, and reducing ‘space’ for infiltrating lymphocytes active (lymphocytic) inflammation. Conversely, ongoing lymphocytic inflammation in anti-CCP positive RA might prevent the occurrence of fibrosis or repair and lead to increased joint destruction. In this retrospective study we were unable to correct for the influence of DMARDs or gender. However, no significant differences were found in DMARD therapy between both groups. The observed difference in gender is not supported in literature by a more aggressive disease or increased occurrence of CCP-antibodies in women with RA.

To our knowledge this is the first study comparing synovial tissue specimens of RA patients with a time interval between biopsies of several years. In keeping with previous reports, our study shows synovial inflammation persists despite DMARD treatment (15) which leads to ongoing progression of joint destruction. We also found an increased expression of MMP-13 in anti-CCP positive patients. This extends our previous finding of a more lymphocytic infiltrate in anti-CCP positive patients as MMP-13 expression is more prominent in lymphocytic synovitis (16). Synovial MMP-13 expression is associated with increased joint destruction in RA (17). Because MMP-13 degrades collagen, a major constituent of fibrosis,
the increased expression might also contribute to the observed difference in fibrosis between anti-CCP positive and –negative patients.

In conclusion, our study shows persistence of synovial inflammation over time with a different progression of synovial inflammation between anti-CCP positive and – negative patients. In anti-CCP positive patients a persistent lymphocytic infiltrate was associated with MMP-13 expression, and progression of joint destruction, while progressive fibrosis is more characteristic of anti-CCP negative disease.
Chapter 7

Reference List


Table 1. Synovial and radiological changes over time.

<table>
<thead>
<tr>
<th></th>
<th>All patients N=30</th>
<th>p-value*</th>
<th>Anti-CCP positive N=18</th>
<th>p-value*</th>
<th>Anti-CCP negative N=12</th>
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<td>2.38 (1.39)</td>
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<td>1.86 (1.09)</td>
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<td>Lymphocytes</td>
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<td>57.7 (39.9)</td>
<td>0.92</td>
<td>72.4 (41.9)</td>
<td>68.6 (44.5)</td>
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<td>Plasmacells</td>
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<td>10.2 (19.3)</td>
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<td>23.7 (36.6)</td>
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<td>Neutrophils</td>
<td>8.6 (26.3)</td>
<td>10.6 (15.9)</td>
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<td>9.3 (31.7)</td>
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<td>Vascularity</td>
<td>22.2 (8.9)</td>
<td>20.4 (9.3)</td>
<td>0.41</td>
<td>24.4 (9.82)</td>
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<td>Fibrosis</td>
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<td>1.33 (1.27)</td>
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<td>Fibrin</td>
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<td>1.80 (1.54)</td>
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<td>0.72 (1.18)</td>
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<td>na</td>
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<td>3</td>
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<td>K/L cl knee</td>
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<td>1.07 (0.9)</td>
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<td>0.8 (0.9)</td>
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<td>11</td>
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Data are means (SD) or absolute number of patients. SLL = synovial lining layer, K/L = Kellgren and Lawrence score. na=not applicable, cl=contralateral * p-values concern testing between both timepoints within a patient group.
Figure 1

Representative images of immunohistological MMP-13 expression in anti-CCP positive (left panel) and anti-CCP negative RA (right panel). Original magnification 100x (upper panel) and 250 X (lower panel).