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Histological Evidence that Infliximab Treatment Leads to Down-regulation of Inflammation and Tissue Remodelling of the Synovial Membrane in Spondyloarthropathy

Extended Report

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Abstract
Objectives. To confirm and extend the immunopathologic evidence of effects of infliximab on synovium in active spondyloarthritis (SpA).

Patients and methods. Synovial biopsies obtained in SpA, at baseline and week 12, were stained and scored by 2 independent observers. Two study populations were evaluated: (I) a cohort of 10 patients, treated with 5 mg/kg infliximab at week 0, 2 and 6, with also 3 placebo-treated patients and (II) a pooled cohort of 20 patients, fulfilling identical inclusion and exclusion criteria, treated with the same loading dose regimen.

Results. In study population I, treatment with infliximab induced reduction of synovial lining layer thickness (p=0.015), endothelial activation (E-selectin, p=0.034) and inflammatory cell infiltration with neutrophils (p=0.041), macrophages (p=0.034), T cells (p=0.026) but not of B and plasma cells, whereas no such trends were observed in the placebo-treated patients. Besides confirming the highly significant down-regulation of inflammation, analysis of cohort II evidenced structural changes such as normalisation of lining layer thickness (p=0.030), reduction in number of blood vessels (p=0.039) and down-regulation of the follicular organisation (p=0.050). No differences in histopathological response were observed between the SpA subtypes.

Conclusions. The present study confirms that profound immunomodulatory changes in the synovium parallel the clinical benefit in SpA patients treated with infliximab independently of the subtype. Moreover, it provides histologic evidence that TNFα blockade does not only down-regulate inflammation but also leads to tissue remodelling.

Key words: Synovium, Spondyloarthritis, Infliximab, TNFα blockade, Histopathology
Introduction

The use of biological therapies that block tumor necrosis factor α (TNFα) has opened new perspectives for the treatment of patients with Spondyloarthropathy (SpA) [1,2,3,4]. Whereas it is now well established that specific intervention in the immune cascade by infliximab results in a remarkable clinical benefit in SpA, preliminary data suggest that it might also have a tissue remodelling effect in SpA [5,6,7,8]. In order to provide more insight in the immunological and biological implications of this therapy, adequate surrogate markers exploring clinical efficacy and paraclinical effects are mandatory. In RA, the use of serial synovial biopsies as a tool in assessing immunopathologic alterations induced by targeted therapies has been well validated [9,10,11,12]. In contrast, in SpA, this methodology of sequential synovial tissue sampling was so far only scarcely used [13,14]. Previously, we explored the impact of infliximab on peripheral synovitis on the histopathological level by serial synovial biopsies in 8 SpA patients [14]). The most striking immunohistopathologic changes included a reduction in lining layer thickness, and a down-regulation of hypervascularity and endothelial activation resulting in a reduction of the inflammatory cell infiltrate with a differential effect on T and B cells.

The aim of the present study was to evaluate the immunopathologic effects of infliximab on the synovial membrane in patients with active SpA, using sequential synovial tissue sampling. Firstly, using an extended panel of immunohistochemical markers we described the synovial membrane before and after infliximab treatment in an independent cohort of SpA patients, and compared these data with the earlier observations as well as with a small placebo cohort. Secondly, since assessing relatively small cohorts of patients is likely to underestimate less prominent immunopathologic changes as well as secondary tissue remodelling, histopathologic data of 20 SpA patients fulfilling identical inclusion and exclusion criteria and who all received the same loading dose regimen of infliximab were pooled and reanalysed. Moreover we analysed in this larger cohort whether SpA subtypes would demonstrate a differential synovial response to infliximab.

Materials and methods

Patients

Study Population I: 10 infliximab-treated and 3 placebo-treated SpA patients

Forty patients with SpA, according to the European Spondyloarthritis Study Group criteria [15], were randomised to receive a loading dose regimen (at week 0, 2 and 6) of 5mg/kg infliximab (n=20) or placebo (n=20) [1]. The initial 12-week placebo-controlled study-period was followed by an open extension phase, in which the placebo-treated group switched to verum treatment. The included patients had active disease, defined by the presence of inflammatory axial pain and/or the presence of peripheral synovitis, were not treated with disease modifying anti-rheumatic drugs and/or steroids, and had stable doses of non-steroidal anti-inflammatory drugs. All patients gave informed consent, as approved by the local Ethical Committee of the Ghent University Hospital. Sequential needle arthroscopy was performed on patients who had been selected from this study based on the presence of knee synovitis in 10 infliximab-treated patients (5 with ankylosing spondylitis (AS), 4 with psoriatic arthritis (PsA), and 1 with undifferentiated SpA (USpA)) and in 3 placebo-treated patients (1 AS, 1 PsA, 1 USpA). Demographic data are summarized in table 1. Data
of global clinical evaluation as well as the evaluation of peripheral arthritis of the patients at baseline and week 12 are shown in table 2.

Table 1: Demographic data of Study Population I (n=10 infliximab-treated, n=3 placebo-treated) and II (n=20): Spondyloarthropathy subtype (Ankylosing Spondylitis / Psoriatic Arthritis / undifferentiated Spondyloarthropathy), sex (male/female), age (median, range), disease duration (median, range)) and presence of HLA B27 (+/-/unknown).

<table>
<thead>
<tr>
<th></th>
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<th>Study Population II</th>
</tr>
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<tr>
<td></td>
<td>Infliximab-treated (n=10)</td>
<td>Placebo-treated (n=3)</td>
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<tr>
<td>SpA subtype (AS / PsA / uSpA)</td>
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<td>1/1/1</td>
</tr>
<tr>
<td>sex (male/female)</td>
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<tr>
<td>age (median (range))</td>
<td>53 (30-66)</td>
<td>54 (44-66)</td>
</tr>
<tr>
<td>disease duration (median (range))</td>
<td>14 (1-42)</td>
<td>39 (10-40)</td>
</tr>
<tr>
<td>HLA B27 (+/-/unknown)</td>
<td>8/2/0</td>
<td>2/1/0</td>
</tr>
</tbody>
</table>

Table 2: Clinical evaluation of the effect of infliximab in Spondyloarthropathy at baseline and week 12 in Study Population I (n=10) and II (n=20): patients assessment of pain, patients assessment of global disease activity, physicians assessment of global disease activity, erythrocyte sedimentation rate, C-reactive protein, patients assessment of pain in peripheral joints, morning stiffness in peripheral joints, tender joint score and swollen joint score. Global and peripheral joint assessments on a 100mm visual analogue scale (VAS). Results are expressed as median (range). P is calculated using the paired Wilcoxon signed rank test.

<table>
<thead>
<tr>
<th></th>
<th>Study Population I (n=10)</th>
<th>Study Population II (n=20)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>week 12</td>
</tr>
<tr>
<td>Patient Pain (VAS)</td>
<td>57 (14-97)</td>
<td>20 (1-39)</td>
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<tr>
<td>Patient Global (VAS)</td>
<td>64 (17-98)</td>
<td>12.5 (0-47)</td>
</tr>
<tr>
<td>Physician Global (VAS)</td>
<td>71 (59-89)</td>
<td>17 (8-31)</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>23.5 (11-101)</td>
<td>6 (1-34)</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>2.3 (0.96-7.42)</td>
<td>0.26 (0-7.93)</td>
</tr>
<tr>
<td>Patient Pain Peripheral Joints (VAS)</td>
<td>55.5 (20-90)</td>
<td>18 (4-40)</td>
</tr>
<tr>
<td>Duration of morning stiffness (min)</td>
<td>235 (0-300)</td>
<td>15 (0-90)</td>
</tr>
<tr>
<td>Tender joint count (number)</td>
<td>10 (3-20)</td>
<td>0.5 (0-6)</td>
</tr>
<tr>
<td>Swollen joint count (number)</td>
<td>5.5 (2-24)</td>
<td>0.5 (0-3)</td>
</tr>
</tbody>
</table>
Study Population II: 20 infliximab-treated SpA patients
Since inclusion criteria, treatment and study protocol and histologic analysis were completely identical in the 8 patients of the open pilot study [16], the 10 patients of the double-blind placebo-controlled study (study population I) and an additional 2 patients included in a compassionate use program, we reanalysed the histopathologic data of this cohort of 20 SpA patients (10 AS, 8 PsA, 2 uSpA) treated for 12 weeks with infliximab (5mg/kg at week 0, 2 and 6). Demographic data for study population II are summarized in table 1

Synovial histopathology
Synovial biopsies were obtained at baseline and week 12 by needle arthroscopy of the knee as described previously [17]. Sixteen synovial biopsies were obtained in each patient. Joint lavage was restricted to a minimum (< 150 ml) and no intra-articular steroids were injected. Synovial biopsies were fixed, stained, and scored as extensively described previously [14,18,19]. Briefly, in each patient 8 paraffin embedded biopsies were stained with hematoxylin and eosin for histological analysis, including mean synovial lining layer thickness, vascularity of the sublining layer, infiltration of the sublining layer, presence of lymphoid aggregates, plasma cells and polymorphonuclear cells. The remaining 8 biopsies were snap frozen and used for immunohistochemistry with the following monoclonal antibodies (Ab): anti-CD146 (endothelial cells, Clone P1H12, Chemicon, Temecula, USA), anti-von Willebrand factor (anti-vWF) (endothelial cells, Clone F8/86, Dako), anti-αVβ3 (integrin expressed on endothelial cells, fibroblasts, osteoclasts…, Clone 23C6, Pharmingen, San Diego, USA), anti-CD3 (T cells, Clone UCHT1, Dako, Glostrup, Denmark), anti-CD4 (T helper cells, Clone MT310, Dako), anti-CD8 (T cytotoxic cells, Clone DK25, Dako), anti-CD19 (B cells, Clone HD37, Dako), anti-CD20 (B cells, Clone L26, Dako), anti-CD38 (plasma cells, Clone AT13/5, Dako), anti-CD138 (plasma cells, Clone CBL455, Chemicon), anti-CD68 (pan-macrophage marker, Clone PG-M1, Dako), anti-CD163 (mature macrophage marker, Clone Ber-MAC3, Dako), anti-CD83 (dendritic cells, Clone HB15A, Immunotech S.A., Marseille, France) and anti-CD1a (interdigitating dendritic cells, Clone NA1/34, Dako), anti-E-selectin (CD62E, endothelial leucocyte adhesion molecule 1 mainly expressed on activated endothelial cells, Clone 1.2B6, Dako), anti-ICAM-1 (CD54, intercellular adhesion molecule 1, Clone 6.5B5, Dako), anti-VCAM-1 (CD106, vascular cell adhesion molecule 1, Clone 1.4C3, Dako). Parallel sections were incubated with irrelevant isotype and concentration matched monoclonal Ab as negative control.

Stained sections were coded and analysed by two independent observers, who were blinded for patient data, time of biopsy sampling (baseline or week 12), treatment schedule (infliximab or placebo) and patient code (sections were not scored by pair). The analysis included all areas of the biopsies and a global score was given for each parameter, using a semiquantitative four-point scale: zero represented the lowest and three the highest level of expression. In case of discordant scores between both observers, the mean of the two scores was used. Since the number of positive cells per synovial section for CD83 and CD1a was too small to be scored semi-quantitatively, this parameter was scored as present or absent.

Statistical analysis
Scores obtained at week 12 were compared with baseline scores using the paired Wilcoxon signed rank test. P-values less than 0.05 were considered to be statistically
significant. In order to minimise the type II error and by this avoiding a reduction of sensitivity, Bonferroni correction was not applied.

Results

Clinical Evaluation

Evaluation of the clinical efficacy of anti-TNFα treatment in SpA in the 12-week placebo-controlled double-blind trial has been described extensively previously, demonstrating a highly significant improvement of global disease activity, inflammatory parameters as well as peripheral synovitis [1]. As shown in Table 2, the infliximab-treated patients of cohort I selected for histologic evaluation of peripheral synovitis behaved similarly to the global group with regard to the baseline values as well as response to therapy, with a clear improvement of global clinical parameters as well as peripheral arthritis at week 12 compared to baseline. At baseline clinical synovitis of the biopsied knee joint was present in all patients, whereas at week 12 only 3/10 patients still had a swollen knee. The 3 placebo-treated patients selected for histologic evaluation behaved clinically similarly as the placebo-cohort reported in the double-blind placebo-controlled trial [1], demonstrating no clinical improvement at week 12 compared to baseline.

Similarly, all 20 patients from study population II showed a significant improvement on all clinical assessments of disease activity at week 12 compared to baseline (Table 2).

Histologic and Immunohistochemical Evaluation of Study Population I

The histologic evaluation of study population I is summarized in table 3 and illustrated in figure 1 and 2.

Synovial lining layer thickness was increased (3-5 cell layers) at baseline in 7/10 patients and was normal (1-2 cell layers) in 9/10 patients at week 12 (p=0.015). The expression of αVβ3 in the lining layer was not altered (p=0.516). A significant reduction in VCAM-1 expression (p=0.034) at week 12 compared to baseline was observed, whereas the expression of ICAM-1 (p=0.238) did not change. Although E-selectin was mainly expressed on endothelial cells, it was also detected in the lining layer. No significant change at week 12 compared to baseline could be observed for E-selectin expression (p=0.180), although this might be due to the fact that E-selectin expression in the lining at baseline was only observed in 3/8 patients; at week 12 this number was reduced to 1/8. There was no alteration of the CD163 expression in the lining layer (p=0.864).

Vascularity tended to diminish with a decrease in 7/10 versus an increase in 2/10 patients (p=0.339). We observed a similar tendency for CD146 (p=0.105) and for von Willebrand factor (p=0.170), with a decrease for both CD146+ and vWF+ blood vessels in 6/8 patients and an increase in only 1/8 patients. Moreover, the expression of αVβ3 in the sublining—a marker for neovascularisation—tended to decrease after treatment with infliximab (p=0.058). The endothelial expression of the different adhesion molecules did not change after therapy with infliximab.

In the sublining, the expression of E-selectin, which probably mimicks non-endothelial cell staining due to vessel sprouting (p=0.034) was significantly reduced after treatment with infliximab. A similar trend for VCAM-1 (p=0.173, with a decrease in 5/8 and an increase in only 2/8 patients) and for ICAM-1 (p=0.141, with a decrease in 5/7 and an increase in only 1/7 patient) was noticed.
The overall degree of inflammatory cell infiltration was reduced at week 12 compared to baseline (p=0.018), with a trend towards reduction of the number of lymphoid aggregates (p=0.073). This was paralleled by a significant reduction of neutrophil infiltration (p=0.041): in 5/10 patients neutrophil infiltration was present at baseline, at week 12 none of the patients exhibited such infiltration. The number of lymphocytes positive for CD3 was decreased at week 12 compared to baseline (p=0.026), with a similar trend for CD4+ (p=0.076) and CD8+ (p=0.061) T cell infiltration. Interestingly, the number of CD19+ lymphocytes tended to decrease (p=0.059), whereas no such trend was observed for CD20+ B cells, CD38+ and CD138+ plasma cells. The number of CD68+ macrophages in the sublining layer was significantly reduced at week 12 (p=0.034), however the number of CD163+ cells was not altered (p=0.469). At baseline in 4/9 patients CD83+ dendritic cells were observed, whereas at week 12 no such cells could be found anymore. Similarly, CD1a+ interdigitating dendritic cells were present in 6/9 patients at baseline, and in only 2/9 patients at week 12.

In contrast with the infliximab-treated patients, none of the histologic characteristics or immunohistochemical markers changed consistently between baseline and week 12 in the placebo cohort.
Table 3: Histologic and immunohistochemical evaluation in Spondyloarthropathy at baseline and week 12 in infliximab-treated patients of Study Population I (n=10). Semiquantitative histologic and immunohistochemical scores are given as median (range). The immunohistochemical markers CD83 and CD1a (sublining layer) were scored as present (+) or absent (-). P-value is calculated for study population I, using the paired Wilcoxon signed rank test for the semiquantitative scores.

<table>
<thead>
<tr>
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<th>Study Population I (n=10 infliximab)</th>
<th>p-value</th>
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<tr>
<td></td>
<td>baseline</td>
<td>week 12</td>
</tr>
<tr>
<td>LINING LAYER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>synovial lining layer thickness</td>
<td>1.5 (1-2)</td>
<td>1 (1-1.5)</td>
</tr>
<tr>
<td>αVβ3 lining</td>
<td>0 (0-3)</td>
<td>1 (0-2.5)</td>
</tr>
<tr>
<td>VCAM-1 lining</td>
<td>3 (1.5-3)</td>
<td>1.5 (1-3)</td>
</tr>
<tr>
<td>ICAM-1 lining</td>
<td>3 (1.5-3)</td>
<td>2.5 (1.5-3)</td>
</tr>
<tr>
<td>E-selectin lining</td>
<td>0 (0-1.5)</td>
<td>0 (0-0.5)</td>
</tr>
<tr>
<td>CD163 lining</td>
<td>2 (0-2.5)</td>
<td>1.5 (0-3)</td>
</tr>
<tr>
<td>BLOODVESSELS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vascularity</td>
<td>1.75 (1-3)</td>
<td>1.25 (1-2.5)</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>3 (0-3)</td>
<td>1.5 (1-3)</td>
</tr>
<tr>
<td>CD146</td>
<td>3 (1-3)</td>
<td>1.5 (0-3)</td>
</tr>
<tr>
<td>αVβ3 endothelial</td>
<td>1 (0-2.5)</td>
<td>0 (0-1.5)</td>
</tr>
<tr>
<td>VCAM-1 endothelial</td>
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<td>0 (0-1.5)</td>
</tr>
<tr>
<td>ICAM-1 endothelial</td>
<td>3 (0-3)</td>
<td>2.5 (1-3)</td>
</tr>
<tr>
<td>E-selectin endothelial</td>
<td>1.5 (0-3)</td>
<td>1 (0-2.5)</td>
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<tr>
<td>SUBLINING LAYER</td>
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<tr>
<td>VCAM-1 sublining</td>
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<td>0 (0-2.5)</td>
</tr>
<tr>
<td>ICAM-1 sublining</td>
<td>3 (0-3)</td>
<td>1.25 (0.5-3)</td>
</tr>
<tr>
<td>E-selectin sublining</td>
<td>2 (0.5-3)</td>
<td>1 (0-2)</td>
</tr>
<tr>
<td>degree of inflammatory cell infiltration</td>
<td>1.75 (0.5-2.5)</td>
<td>0.75 (0-2)</td>
</tr>
<tr>
<td>number of neutrophils</td>
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<td>0 (0-0)</td>
</tr>
<tr>
<td>number of lymphoid aggregates</td>
<td>1.75 (0-3)</td>
<td>0.5 (0-3)</td>
</tr>
<tr>
<td>CD3</td>
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</tr>
<tr>
<td>CD4</td>
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</tr>
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<td>CD8</td>
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<tr>
<td>number of plasma cells</td>
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<td>CD38</td>
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<td>CD138</td>
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</tr>
<tr>
<td>CD68</td>
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<td>1 (0.5-2)</td>
</tr>
<tr>
<td>CD163</td>
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<td>0.5 (0-2.5)</td>
</tr>
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<td>CD83 (+/-)</td>
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<td>0/9</td>
</tr>
<tr>
<td>CD1a (+/-)</td>
<td>6/9</td>
<td>2/9</td>
</tr>
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</table>

Histologic and Immunohistochemical Evaluation of Study Population II
The data are summarized in Table 4.
At baseline, lining layer hyperplasia was present in 11/20 patients, whereas after infliximab therapy, 19/20 patients had a normal lining layer thickness (p=0.003). The expression of αVβ3 in the lining layer was not modified (p=0.856). CD163 expression showed a trend towards reduction with a decrease in 10/20 patients, status quo in 6/20 and an increase in only 4/20 (p=0.061).
Vascularity was significantly decreased at week 12 compared to baseline (p=0.039), which was paralleled by a down-regulation of the neovascularisation marker αVβ3 (p=0.024). Evaluation of the endothelial expression of the adhesion molecules revealed a significant down-regulation of VCAM-1 at week 12, when compared to baseline (p=0.018), and a similar trend for E-selectin (p=0.083, with a decrease in 9/18, and an increase in only 3/18; in the 5/6 patients with no change, baseline values were already zero). Endothelial expression of ICAM-1 did not change after treatment with infliximab (p= 1.000).

When evaluating the expression of adhesion molecules in the sublining, a significant down-regulation was found for VCAM-1 (p=0.047), ICAM-1 (p=0.007) as well as for E-selectin (p=0.006).

Overall inflammatory cell infiltration (p=0.017) as well as the presence of lymphoid aggregates (p=0.050), were both reduced at week 12. This coincided with a significant reduction in the number of neutrophils (p=0.005), CD4+ (p=0.004) and CD8+ (p=0.013) T cells as well as CD68+ (p=0.002) and CD163+ (p=0.050) macrophages. A similar trend was found for CD3+ T cells (p=0.073). However no change in numbers of CD20+ B cells (p=0.468) or plasma cells (p=0.749) could be observed.

Table 4: Histological and immunohistochemical evaluation of the effect of infliximab (5mg/kg IV at week 0, Week 2 and week 6) on the synovial membrane in Study Population II (n=20). Results are expressed as median (range). P-value is calculated using the paired Wilcoxon signed rank test.
Discussion
The present study analysed an independent cohort of 10 infliximab-treated SpA patients, in which baseline synovial tissue samples depicted characteristics suggestive for SpA synovitis: a moderate lining hyperplasia, a strong hypervascularisation with endothelial activation, and a moderate and diffuse inflammatory infiltration with macrophages, as well as lymphocytes and polymorphonuclear cells [18,20]. After 12 weeks of infliximab treatment, the earlier reported effects such as the reduction of synovial lining layer thickness, endothelial activation, and inflammatory cell infiltration with polymorphonuclear cells, macrophages, T cells but not B and plasma cells were confirmed [14]. In addition, the panel of immunohistochemic markers was extended by specific markers for vascularity (vWF, CD146), B-cells (CD19), plasma cells (CD38, CD138) and dendritic cells (CD1a, CD83). These stainings confirmed that there was no significant decrease in B cells or plasma cells. Whereas a slight trend to decrease for CD19 but not for the other B cell markers is noted, these data essentially indicate that the effect of infliximab on the B cell lineage is less pronounced than on the T lymphocytes, macrophages and PMN. The additional stainings also indicate a significant decrease in vascularity, as reflected by a down-regulation of vWF and CD146 expression. Furthermore, we provided new evidence for a significant decrease of the follicular organisation of the residual inflammatory infiltration, which was paralleled by a decrease of follicular dendritic cells as identified by CD1a and CD83. Another new indication of the present study is that these findings in infliximab-treated patients contrasted with histopathologic findings in 3 placebo-treated patients, which showed no tendency at all towards reduction of the synovial inflammation, on the contrary scores varied in both senses. This indicates that the observed changes in the treated group are not biased by the needle arthroscopy, the biopsy sampling procedure, nor the analysis methodology, as is previously demonstrated in RA [21].

Beside the fact that the present study confirmed and extended our pilot observations [14], another important aspect is that both studies were conducted in exactly the same way (patient inclusion, treatment regimen, biopsy sampling, histological analysis), so that the data can be pooled and analysed together. Indeed, a major drawback of this kind of approach is the relatively small number of observations: significant changes observed in a small cohort probably reflect genuine phenomena, as evidenced by the present confirmation study, but discrete alterations in synovial histopathology may be overlooked more easily, including putative alterations that would be observed in only a subgroup of the SpA population. Therefore, we pooled the histopathologic data of 20 SpA patients. The analysis of this pooled cohort generated some new important conclusions. The trend towards a down-regulation of the endothelial expression of αVβ3 and VCAM-1, the expression in the sublining of ICAM-1, VCAM-1 as well as the presence of lymphoid aggregates, CD4+ and CD8+ cells, reached significance in study population II. Although CD68 and CD163 are both expressed by macrophages, they do not identify the same cell populations: CD68 is a pan-macrophage marker, whereas CD163 is expressed on activated macrophages in an advanced maturation stage [22,23]. Moreover, recent evidence indicates that CD163 does not only identify a separate macrophage subpopulation, but also confers to specific functional capacities, which may be important in the pathogenesis of SpA [24]. Although in patient cohort I we could not observe a down-regulation of CD163, after pooling the data a consistent trend towards a decrease of CD163 in the lining and sublining was noticed (resp. p=0.061 and p=0.050) and this in accordance with the findings of a significant down-regulation of CD68 positive cells.
Not only a highly significant down-regulation of immune activation and inflammation could be demonstrated in this larger cohort, but these changes also resulted in a trend towards normalisation of the histology of the synovial membrane. Structural changes involving normalisation of lining layer thickness and significant down-regulation of the number of blood vessels witnesses this trend and substantiate that interfering with TNFα not only modulates inflammation, but can also restore the tissue architecture. Whether these changes would lead towards a complete normalisation should be studied by evaluating synovial biopsies sampled after a longer period of infliximab treatment. On the other hand, our recent observations of profound down-modulation of MMP’s in SpA synovium by infliximab treatment could fit in the hypothesis that structural remodelling of the synovium could precede structural changes in bone and cartilage [25]. This would substantiate some of the preliminary observations made in patients with psoriatic arthritis in whom remission was paralleled by the radiological observation of remodelling of destructed finger joints [8,26].

Beside the demonstration of tissue remodelling, this pooled cohort of 20 patients also allowed for the first time to explore if subtle differences could be present in synovial histology between the SpA subtypes and/or in histological response to treatment. The comparison of the immunohistopathologic response between the subtypes AS(n=10) and PsA(n=8) did not reveal any significant differences (results not shown). Even though the number of patients in the subgroups was small, SpA subtypes as such do not appear to demonstrate a highly discriminative immunohistopathologic architecture at baseline [27]. Moreover, the synovial evaluation after infliximab therapy was in accordance with the observation that the clinical response between the subtypes is not significantly different either, underscoring the overall similarity of the different SpA subsets as far as peripheral synovitis and response to infliximab treatment is concerned (unpublished data).

In summary, the similar findings in two independent infliximab-treated cohorts (but not in placebo patients) and the fact that the histologic analysis confirms the clinical benefit of infliximab in SpA indicate the potential of sequential synovial tissue analysis as surrogate marker in the evaluation of targeted therapies in SpA. In addition, the observation of down-modulation of inflammation as well as structural remodelling of the synovium warrants longer-term prospective evaluation of structural repair by TNFα blockade by a combined histological and radiological approach. Finally, the present study did not provide evidence that peripheral synovitis in different SpA subtypes responds differentially to infliximab treatment.

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References


Figure 1: Immunomodulatory effect of infliximab (A= study population I, n = 10) or placebo (B= study population I, n = 3) on synovial histology in patients with active spondyloarthropathy. Synovial biopsies obtained at week 0 and week 12, were scored on a semiquantitative scale (0-3) by two independent observers (p-value calculated with the paired Wilcoxon ranks sign test for study population I). Representative sections of the evaluation at baseline (C) and at week 12 (D) in infliximab-treated patients (study population I) are shown and the corresponding semiquantitative score for each picture is indicated. The evaluated parameters included: a) degree of inflammatory cell infiltration (score 2.5 at baseline, score 0.5 at week 12), b) number of neutrophils (score 3 at baseline, score 0 at week 12), c) number of CD3+ T cells (score 2 at baseline, score 0 at week 12), d) number of CD20+ B cells (score 0 at baseline, score 2 at week 12), f) number of CD38+ plasma cells (score 3 at baseline, score 3 at week 12), and g) number of CD68+ macrophages (score 2 at baseline, score 1 at week 12).

Figure 2: Tissue remodelling effect of infliximab (A= study population I, n = 10) or placebo (B= study population I, n = 3) on synovial histology in patients with active spondyloarthropathy. Synovial biopsies obtained at week 0 and week 12, were scored on a semiquantitative scale (0-3) by two independent observers (p-value calculated with the paired Wilcoxon ranks sign test for study population I). Representative sections of the evaluation in infliximab-treated patients (study population I) at baseline (C) and at week 12 (D) are shown and the corresponding semiquantitative score for each picture is indicated. The evaluated parameters included: a) synovial lining layer thickness (score 2 at baseline, score 1 at week 12), b) degree of vascularity (score 3 at baseline, score 1 at week 12), c) number of CD146+ endothelial cells (score 3 at baseline, score 1 at week 12), d) endothelial expression of von Willebrand factor (score 3 at baseline, score 1 at week 12), e) degree of follicular formation (score 2 at baseline, score 1.5 at week 12), and e) presence of CD83+ dendritic cells (present at baseline, absent at week 12).
**Figure 1**

(A) Cellular Infiltration

(B) Neutrophils

(C) CD3

(D) CD20

(E) CD38

(F) CD68

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Cellular Infiltration

- Neutrophils
- CD3
- CD20
- CD38
- CD68

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We observed statistically significant differences in cellular infiltration and specific immune cell populations between groups 0 and 12 weeks post-treatment. The p-values for each comparison are as follows:

- **Cellular Infiltration (A)**: p=0.018
- **Neutrophils (B)**: p=0.041
- **CD3 (C)**: p=0.026
- **CD20 (D)**: p=0.750
- **CD38 (E)**: p=0.516
- **CD68 (F)**: p=0.034

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These findings suggest a significant increase in cellular infiltration and specific immune cell populations in the treated group compared to the control group.
Figure 2
Histological evidence that infliximab treatment leads to downregulation of inflammation and tissue remodelling of the synovial membrane in spondyloarthropathy

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