EXTENDED REPORT

Histological evidence that infliximab treatment leads to downregulation of inflammation and tissue remodelling of the synovial membrane in spondyloarthropathy

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Objective: To confirm and extend the immunopathological evidence of effects of infliximab on the synovium in active spondyloarthropathy.

Methods: Synovial biopsies obtained in patients with spondyloarthropathy at baseline and week 12 were stained and scored by two 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, plus three placebo treated patients; and II, a pooled cohort of 20 patients fulfilling identical inclusion and exclusion criteria and treated with the same loading dose regimen.

Results: In study population I, treatment with infliximab induced reduction in 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), and T cells (p = 0.026), but not with B cells and plasma cells; no such trends were observed in the placebo treated patients. Besides confirming the highly significant downregulation of inflammation, analysis of cohort II showed structural changes such as normalisation of lining layer thickness (p = 0.030), reduction in the number of blood vessels (p = 0.039), and downregulation of follicular organisation (p = 0.050). No differences in histopathological response were observed between spondyloarthropathy subtypes.

Conclusions: Profound immunomodulatory changes in the synovium parallel the clinical benefit in patients with spondyloarthropathy treated with infliximab, independently of the subtype. The study provides histological evidence that TNFα blockade not only downregulates inflammation but also leads to tissue remodelling.

T he use of biological treatments that block tumour necrosis factor α (TNFα) has opened new perspectives for the treatment of patients with spondyloarthropathy.[1–4] While it is now well established that specific intervention in the immune cascade by infliximab results in remarkable clinical benefit in spondyloarthropathy, preliminary data suggest that it might also have a tissue remodelling effect in this condition.[5–8]

To gain more insight in the immunological and biological implications of this treatment, it is mandatory to have adequate surrogate markers exploring clinical efficacy and paraclinical effects. In rheumatoid arthritis, the use of serial synovial biopsies as a tool in assessing immunopathological alterations induced by targeted treatments has been well validated.[9–12] In contrast, in spondyloarthropathy, this methodology of sequential synovial tissue sampling has so far scarcely been used.[13 14] In a previous study, we explored the impact of infliximab on peripheral synovitis at the histopathological level by serial synovial biopsies in eight patients with spondyloarthropathy.[14] The most striking immunohistopathological changes included a reduction in lining layer thickness and downregulation of hypervascularity and endothelial activation, resulting in a reduction in the inflammatory cell infiltrate with a differential effect on T and B cells.

Our aim in the present study was to evaluate the immunopathological effects of infliximab on the synovial membrane in patients with active spondyloarthropathy, employing sequential synovial tissue sampling. First, using an extended panel of immunohistochemical markers, we

described the synovial membrane before and after infliximab treatment in an independent cohort of patients, and compared these data with the earlier observations as well as with a small placebo cohort. Second, as the assessment of relatively small cohorts is likely to underestimate less prominent immunopathological changes and secondary tissue remodelling, histopathological data on 20 patients fulfilling identical inclusion and exclusion criteria and all receiving the same loading dose regimen of infliximab were pooled and reanalysed. In this larger cohort we examined whether spondyloarthropathy subtypes show a differential synovial response to infliximab.

METHODS

Patients

Group I: 10 infliximab treated and three placebo treated patients

Forty patients with spondyloarthropathy according to the European Spondyloarthropathy Study Group criteria[15] were randomised to receive a loading dose regimen (at week 0, 2, and 6) of 5 mg/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 active treatment. The patients who were included had active disease, defined by the presence of inflammatory axial pain or peripheral synovitis, were not treated with disease modifying antirheumatic drugs or steroids, and had stable doses of non-steroidal anti-inflammatory drugs.

Table 1 Demographic data on study populations I and II (n = 20)

	Study population I		Study population II (n = 20)
Variable	Infliximab treated (n = 10)	Placebo treated (n = 3)	
SpA subtype (AS/PsA/uSpA)	5/4/1	1/1/1	10/8/2
Sex (male/female)	8/2	3/0	16/4
Age (years) (median (range))	53 (30 to 66)	54 (44 to 66)	49 (38 to 66)
Disease duration (median (range))	14 (1 to 42)	39 (10 to 40)	18.5 (1 to 43)
HLA B27 (+/−/unknown)	8/2/0	2/1/0	13/5/2

Values are n or median (range).
Spondyloarthropathy subtype: AS, ankylosing spondylitis; PsA, psoriatic arthritis; uSpA, undifferentiated spondyloarthropathy.
HLA, human leucocyte antigen; SpA, spondyloarthropathy.

All patients gave their informed consent, as approved by the local ethics committee of the Ghent University Hospital.

For the present study, sequential needle arthroscopy was undertaken on patients selected from the main study on the basis of the presence of knee synovitis in 10 infliximab treated patients (five with ankylosing spondylitis, four with psoriatic arthritis, and one with undifferentiated spondyloarthropathy) and in three placebo treated patients (one with ankylosing spondylitis, one with psoriatic arthritis, and one with undifferentiated spondyloarthropathy). Demographic data are summarised in table 1. Data on global clinical evaluation as well as on the evaluation of peripheral arthritis in the patients at baseline and week 12 are shown in table 2.

Group II: 20 infliximab treated patients

As the inclusion criteria, treatment and study protocols, and histological analyses were identical in the eight patients of the open pilot study,[16] the 10 patients of the double blind placebo controlled study (study population I), and an additional two patients included in a compassionate use programme, we reanalysed the histopathological data of this cohort of 20 spondyloarthropathy patients (10 with ankylosing spondylitis, eight with psoriatic arthritis, and two with undifferentiated spondyloarthropathy) treated for 12 weeks with infliximab (5 mg/kg at week 0, 2, and 6). Demographic data for study population II are summarised 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.[14 18 19] Briefly, in

each patient eight paraffin embedded biopsies were stained with haematoxylin and eosin for histological analysis, including mean synovial lining layer thickness, vascularity of the sublining layer, infiltration of the sublining layer, and presence of lymphoid aggregates, plasma cells, and poly-morphonuclear cells. The remaining eight biopsies were snap frozen and used for immunohistochemistry with the following monoclonal antibodies: anti-CD146 (endothelial cells, clone P1H12; Chemicon, Temecula, California, USA), anti-von Willebrand factor (anti-vWF) (endothelial cells, clone F8/86; Dako, Glostrup, Denmark), anti-αVβ3 (integrin expressed on endothelial cells, fibroblasts, osteoclasts…, clone 23C6; Pharmingen, San Diego, California, USA), anti-CD3 (T cells, clone UCHT1; Dako), 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 SA, Marseille, France), 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), and anti-VCAM-1 (CD106, vascular cell adhesion molecule 1, clone 1.4C3; Dako). Parallel sections were incubated with irrelevant isotype and concentration matched monoclonal antibody 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

Table 2 Clinical evaluation of the effect of infliximab on spondyloarthropathy at baseline and week 12 in study populations I and II

	Study population I (n = 10)			Study population II (n = 20)		
Variable	Baseline	Week 12	p Value*	Baseline	Week 12	p Value*
Patient pain (VAS)	57 (14 to 97)	20 (1 to 39)	0.005	69 (14 to 100)	16 (1 to 86)	<0.001
Patient global (VAS)	64 (17 to 98)	12.5 (0 to 47)	0.005	69 (17 to 100)	15 (0 to 73)	<0.001
Physician global (VAS)	71 (59 to 89)	17 (8 to 31)	0.005	64 (35 to 89)	15 (8 to 75)	<0.001
ESR (mm/h)	23.5 (11 to 101)	6 (1 to 34)	0.007	28 (11 to 101)	7 (1 to 59)	<0.001
C reactive protein (mg/dl)	2.3 (0.96 to 7.42)	0.26 (0 to 7.93)	0.047	3.4 (1.0 to 29.0)	0.3 (0 to 7.9)	0.001
Patient pain peripheral joints (VAS)	55.5 (20 to 90)	18 (4 to 40)	0.007	64 (20 to 99)	18 (0 to 84)	<0.001
Duration of morning stiffness (min)	235 (0 to 300)	15 (0 to 90)	0.008	133 (0 to 300)	10 (1 to 90)	<0.001
Tender joint count (n)	10 (3 to 20)	0.5 (0 to 6)	0.007	10 (1 to 20)	0 (0 to 6)	<0.001
Swollen joint count (n)	5.5 (2 to 24)	0.5 (0 to 3)	0.008	7 (0 to 24)	1 (0 to 7)	<0.001

Results are expressed as median (range).
Global and peripheral joint assessments on a 100 mm visual analogue scale.
*p Values calculated using the paired Wilcoxon signed rank test.
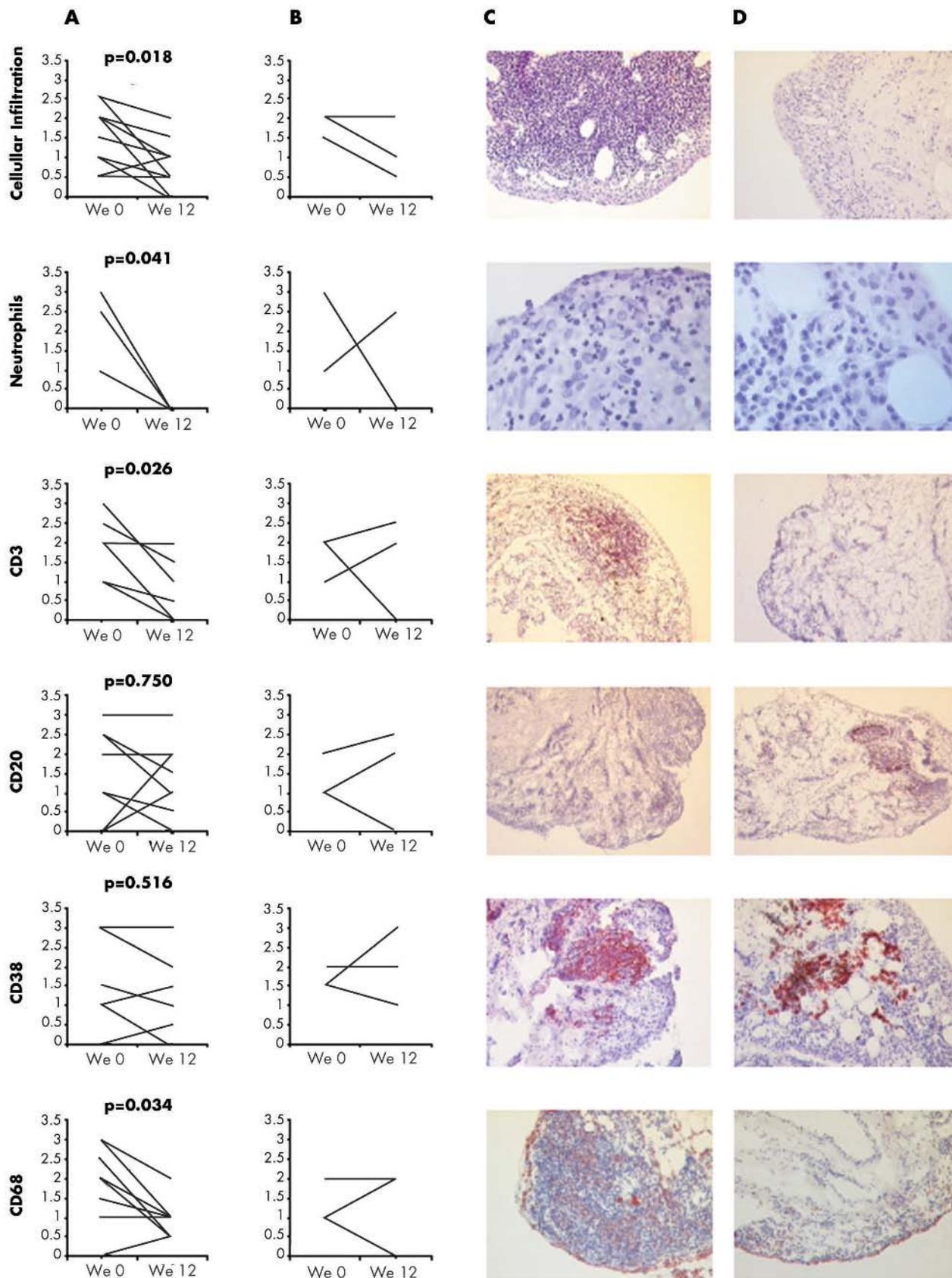ESR, erythrocyte sedimentation rate; VAS, visual analogue scale.

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 using the paired Wilcoxon signed ranks 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 variables evaluated included: the degree of inflammatory cell infiltration (score 2.5 at baseline, score 0.5 at week 12); the number of neutrophils (score 3 at baseline, score 0 at week 12); the number of CD3+ T cells (score 2 at baseline, score 0 at week 12); the number of CD20+ B cells (score 0 at baseline, score 2 at week 12); the number of CD38+ plasma cells (score 3 at baseline, score 3 at week 12); and the 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 using the paired Wilcoxon signed ranks 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 variables evaluated included: the synovial lining layer thickness (score 2 at baseline, score 1 at week 12); the degree of vascularity (score 3 at baseline, score 1 at week 12); the number of CD146+ endothelial cells (score 3 at baseline, score 1 at week 12); endothelial expression of von Willebrand factor (score 3 at baseline, score 1 at week 12); the degree of follicular formation (score 2 at baseline, score 1,5 at week 12); and the presence of CD83+ dendritic cells (present at baseline, absent at week 12). NC, not calculable.

Table 3 Histological and immunohistochemical evaluation in spondyloarthropathy at baseline and week 12 in infliximab treated patients of study population I (n = 10)

	Study population I (n = 10 infliximab)		
	Baseline	Week 12	p Value
Lining layer			
Synovial lining layer thickness	1.5 (1 to 2)	1 (1 to 1.5)	0.015
αVβ3 lining	0 (0 to 3)	1 (0 to 2.5)	0.516
VCAM-1 lining	3 (1.5 to 3)	1.5 (1 to 3)	0.034
ICAM-1 lining	3 (1.5 to 3)	2.5 (1.5 to 3)	0.238
E-selectin lining	0 (0 to 1.5)	0 (0 to 0.5)	0.180
CD163 lining	2 (0 to 2.5)	1.5 (0 to 3)	0.864
Blood vessels			
Vascularity	1.75 (1 to 3)	1.25 (1 to 2.5)	0.339
von Willebrand factor	3 (0 to 3)	1.5 (1 to 3)	0.170
CD146	3 (1 to 3)	1.5 (0 to 3)	0.105
αVβ3 endothelial	1 (0 to 2.5)	0 (0 to 1.5)	0.058
VCAM-1 endothelial	0 (0 to 1.5)	0 (0 to 1.5)	0.180
ICAM-1 endothelial	3 (0 to 3)	2.5 (1 to 3)	1.000
E-selectin endothelial	1.5 (0 to 3)	1 (0 to 2.5)	0.230
Sublining layer			
VCAM-1 sublining	1 (0 to 3)	0 (0 to 2.5)	0.173
ICAM-1 sublining	3 (0 to 3)	1.25 (0.5 to 3)	0.141
E-selectin sublining	2 (0.5 to 3)	1 (0 to 2)	0.034
Degree of inflammatory cell infiltration	1.75 (0.5 to 2.5)	0.75 (0 to 2)	0.018
Number of neutrophils	0.5 (0 to 3)	0 (0 to 0)	0.041
Number of lymphoid aggregates	1.75 (0 to 3)	0.5 (0 to 3)	0.073
CD3	2 (0 to 3)	0.5 (0 to 2)	0.026
CD4	1.5 (0 to 3)	0.5 (0 to 2)	0.076
CD8	2 (0 to 2.5)	1 (0 to 1)	0.061
CD19	1 (0 to 3)	0 (0 to 1)	0.059
CD20	1.5 (0 to 3)	1 (0 to 3)	0.750
Number of plasma cells	0.5 (0 to 3)	0 (0 to 3)	0.258
CD38	1.5 (0 to 3)	1.5 (0 to 3)	0.516
CD138	1 (0 to 3)	1 (0 to 3)	0.680
CD68	2 (0 to 3)	1 (0.5 to 2)	0.034
CD163	1.25 (0 to 3)	0.5 (0 to 2.5)	0.469
CD83 (+/−)	4/9	0/9	NC
CD1α (+/−)	6/9	2/9	0.125

Semiquantitative histological and immunohistochemical scores are given as median (range). The immunohistochemical markers CD83 and CD1α (sublining layer) were scored as present (+) or absent (−). The p value was calculated for study population I using the paired Wilcoxon signed rank test for the semiquantitative scores and the McNemar test for the dichotomous scores.
NC, not calculable.

were not scored by pair). The analysis included all areas of the biopsies and a global score was given for each variable, using a semiquantitative four point scale: 0 represented the lowest and 3 the highest level of expression. In case of discordant scores between the two observers, the mean of the two scores was used. As the number of positive cells per synovial section for CD83 and CD1a was too small to be scored semiquantitatively, this variable 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. Probability (p) values less than 0.05 were considered statistically significant. In order to minimise the type II error and thus avoid a reduction in sensitivity, the Bonferroni correction was not applied.

RESULTS
Clinical evaluation

Evaluation of the clinical efficacy of anti-TNFα treatment in spondyloarthropathy in the 12 week, placebo controlled, double blind trial has been described extensively before, results demonstrating a highly significant improvement in global disease activity, inflammatory indices, and peripheral synovitis.[1] As shown in table 2, the infliximab treated patients of cohort I selected for histological evaluation of

peripheral synovitis behaved in a similar way to the global group with regard to the baseline values and response to treatment, with a clear improvement in global clinical indices as well as peripheral arthritis by week 12 compared with baseline. At baseline, clinical synovitis of the biopsied knee joint was present in all patients, whereas by week 12 only three of the 10 patients still had a swollen knee. The three placebo treated patients selected for histological evaluation behaved clinically in a similar way to the placebo cohort reported in the double blind, placebo controlled trial,[1] with no clinical improvement at week 12 compared with baseline.

All 20 patients from study population II showed significant improvement on all clinical assessments of disease activity by week 12 compared with baseline (table 2).

Histological and immunohistochemical evaluation of study population I

The histological evaluation of study population I is summarised in table 3 and illustrated in figs 1 and 2.

Synovial lining layer thickness was increased (three to five cell layers) at baseline in seven of the 10 patients and was normal (one to two cell layers) in nine patients at week 12 (p = 0.015). Expression of αVβ3 in the lining layer was not altered (p = 0.516). A significant reduction in VCAM-1 expression (p = 0.034) was observed at week 12 compared with baseline, whereas the expression of ICAM-1 (p = 0.238) did not change. Although E-selectin was mainly expressed on

Table 4 Histological and immunohistochemical evaluation of the effect of infliximab (5 mg/kg intravenously at week 0, week 2, and week 6) on the synovial membrane in study population II (n = 20)

Variable	Study population II (n = 20 infliximab)		
	Baseline	Week 12	p Value
Lining layer			
Synovial lining layer thickness	1.5 (1 to 2)	1 (1 to 1.5)	0.003
αVβ3 lining	0 (0 to 3)	0 (0 to 2.5)	0.856
CD163 lining	2 (0 to 3)	1.25 (0 to 3)	0.061
Blood vessels			
Vascularity	2 (1 to 3)	1.5 (0.5 to 3)	0.039
αVβ3 endothelial	0 (0 to 2.5)	0 (0 to 1.5)	0.024
E-selectin endothelial	1 (0 to 3)	0.5 (0 to 2.5)	0.083
ICAM-1 endothelial	2 (0 to 3)	2 (1 to 3)	1.000
VCAM-1 endothelial	0 (0 to 2.5)	0 (0 to 1.5)	0.018
Sublining layer			
E-selectin sublining	1 (0 to 3)	0 (0 to 2)	0.006
ICAM-1 sublining	2.5 (0 to 3)	1 (0 to 3)	0.007
VCAM-1 sublining	2 (0 to 3)	1 (0 to 3)	0.047
Degree of inflammatory cell infiltration	1.75 (0 to 3)	1 (0 to 2.5)	0.017
Number of neutrophils	0.5 (0 to 3)	0 (0 to 0.5)	0.005
Number of lymphoid aggregates	0.25 (0 to 3)	0 (0 to 3)	0.050
CD3	1 (0 to 3)	1 (0 to 3)	0.073
CD4	1 (0 to 3)	0.5 (0 to 2)	0.004
CD8	1 (0 to 3)	1 (0 to 3)	0.013
CD20	1 (0 to 3)	1 (0 to 3)	0.486
Number of plasma cells	0 (0 to 3)	0.25 (0 to 3)	0.749
CD68	1.5 (0 to 3)	0.5 (0 to 2)	0.002
CD163 sublining	1.25 (0 to 3)	0.5 (0 to 2.5)	0.050

Results are expressed as median (range). The p values were calculated using the paired Wilcoxon signed rank test.

endothelial cells, it was also detected in the lining layer. No significant change in E-selectin expression could be observed at week 12 compared with baseline (p = 0.180), although this might be because E-selectin expression in the lining at baseline was only observed in three of eight patients; by week 12 this number was reduced to one of eight. There was no alteration in CD163 expression in the lining layer (p = 0.864).

Vascularity tended to diminish, with a decrease in seven of the 10 patients and an increase in two (p = 0.339). We observed a similar tendency for CD146 (p = 0.105) and von Willebrand factor (p = 0.170), with a decrease in both CD146+ and vWF+ blood vessels in six of eight patients and an increase in only one. Moreover, the expression of αVβ3 in the sublining—a marker for neovascularisation— tended to decrease after treatment with infliximab (p = 0.058). Endothelial expression of the different adhesion molecules did not change after infliximab treatment.

In the sublining, expression of E-selectin, which probably mimics non-endothelial cell staining caused by vessel sprouting (p = 0.034), was significantly reduced after treatment with infliximab. A similar trend for VCAM-1 was found (p = 0.180, with a decrease in five of eight patients and an increase in only two) and for ICAM-1 (p = 0.141, with a decrease in five and an increase in one).

The overall degree of inflammatory cell infiltration was reduced at week 12 compared with baseline (p = 0.018), with a trend towards reduction in the number of lymphoid aggregates (p = 0.073). This was paralleled by a significant reduction in neutrophil infiltration (p = 0.041): in five of 10 patients neutrophil infiltration was present at baseline, but by week 12 none of the patients showed such infiltration. The number of lymphocytes positive for CD3 was decreased at week 12 compared with baseline (p = 0.026), with a similar

trend for CD4+ (p = 0.076) and CD8+ (p = 0.061) T cell infiltration. The number of CD19+ lymphocytes tended to decrease (p = 0.059), whereas no such trend was observed for CD20+ B cells or 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 four of nine patients CD83+ dendritic cells were observed, whereas by week 12 such cells could no longer be found. Similarly, CD1a+ interdigitating dendritic cells were present in six of nine patients at baseline, and in only two patients by week 12.

In contrast to the infliximab treated patients, none of the histological characteristics or immunohistochemical markers changed consistently between baseline and week 12 in the placebo cohort.

Histological and immunohistochemical evaluation of study population II

The data are summarised in table 4.

At baseline, lining layer hyperplasia was present in 11 of 20 patients, whereas after infliximab treatment, 19 of 20 patients had a normal lining layer thickness (p = 0.030). 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 of the 20 patients, unchanged in six, and increased in only four (p = 0.061).

Vascularity was significantly decreased at week 12 compared with baseline (p = 0.039), and this was paralleled by downregulation of the neovascularisation marker αVβ3 (p = 0.024). Evaluation of the endothelial expression of the adhesion molecules revealed a significant downregulation of VCAM-1 at week 12 compared with baseline (p = 0.018), and a similar trend for E-selectin (p = 0.083, with a decrease in nine of 18 patients and an increase in only three; in five of the six 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, significant downregulation was found for VCAM-1 (p = 0.047), ICAM-1 (p = 0.007), and E-selectin (p = 0.006).

Overall inflammatory cell infiltration (p = 0.017) and the presence of lymphoid aggregates (p = 0.050) were both reduced by 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, and 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) was found.

DISCUSSION

In this study we analysed an independent cohort of 10 infliximab treated spondyloarthropathy patients in whom baseline synovial tissue samples had characteristics suggestive of spondyloarthropathy synovitis: moderate lining hyperplasia, strong hypervascularisation with endothelial activation, and moderate and diffuse inflammatory infiltration with macrophages, lymphocytes, and polymorphonuclear cells.[18] [20] After 12 weeks of infliximab treatment, the previously reported effects were confirmed—that is, reduction in synovial lining layer thickness, endothelial activation, and inflammatory cell infiltration with polymorphonuclear cells, macrophages, and T cells but not B cell and plasma cells.[14] In addition, the panel of immunohistochemical 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. While we noted a slight trend for CD19 (but not the other B cell markers) to decrease, these data essentially indicate that the effect of infliximab on the B cell lineage is less pronounced than on the T lymphocytes, macrophages, and neutrophils.

The additional stainings also indicate a significant decrease in vascularity, as reflected by a downregulation of vWF and CD146 expression. Furthermore, we provided new evidence for a decrease in the follicular organisation of the residual inflammatory infiltration, which was paralleled by a decrease in 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 histopathological findings in three placebo treated patients, in whom there was no tendency at all towards reduction in the synovial inflammation; on the contrary, the scores varied in both directions. This indicates that the observed changes in the treated group were not biased by the needle arthroscopy, the biopsy sampling procedure, or the analysis methodology, as has previously been shown in rheumatoid arthritis.[21]

Besides 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, and 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 readily. These include putative alterations that would be observed in only a subgroup of the spondyloarthropathy population. We therefore pooled the histopathological data on 20 spondyloarthropathy patients. The analysis of this pooled cohort generated some important new conclusions: the trend towards downregulation of the endothelial expression of αVβ3 and VCAM-1, expression in the sublining of ICAM-1 and VCAM-1, and the presence of lymphoid aggregates and 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 not only identifies a separate macrophage subpopulation, but also confers specific functional capacities which may be important in the pathogenesis of spondyloarthropathy.[24] Although we did not observe downregulation of CD163 in patient cohort I, after pooling the data a consistent trend towards a decrease in CD163 in the lining and sublining was noticed (p = 0.061 and p = 0.050, respectively), and this in accordance with the finding of downregulation of CD68 positive cells.

Not only could a highly significant downregulation of immune activation and inflammation 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 downregulation of the number of blood vessels were witness to this trend and confirmed that interfering with TNFα not only modulates inflammation but can also restore the tissue architecture. Whether these changes can lead to 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 downmodulation of matrix metalloproteinases in spondyloarthropathic

synovium by infliximab treatment could fit with the hypothesis that structural remodelling of the synovium precedes 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 damaged finger joints.[8][26]

Beside the demonstration of tissue remodelling, this pooled cohort of 20 patients also allowed us to explore whether there are subtle differences in synovial histology between the spondyloarthropathy subtypes or in responses to treatment. The comparison of the immunohistopathological response between the subtypes ankylosing spondylitis (n = 10) and psoriatic arthritis (n = 8) did not reveal any significant differences (results not shown). Though the number of patients in the subgroups was small, spondyloarthropathy subtypes as such do not appear to show a highly discriminative immunohistopathological architecture at baseline.[27] Moreover, the synovial evaluation after infliximab treatment 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 spondyloarthropathy subsets as far as peripheral synovitis and response to inflixmab treatment is concerned (unpublished data).

Conclusions

The similar findings in two independent infliximab treated cohorts (but not in placebo patients), and the fact that the histological analysis confirmed the clinical benefit of infliximab in spondyloarthropathy, indicate the potential of sequential synovial tissue analysis as a surrogate marker in evaluating targeted treatments in spondyloarthropathy. In addition, the observation of downmodulation of inflammation as well as structural remodelling of the synovium warrants a longer term prospective evaluation of structural repair by TNFα blockade, by a combined histological and radiological approach. Finally, our study provided no evidence that peripheral synovitis in different spondyloarthropathy subtypes responds differentially to infliximab treatment.

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