

## Identification of Synovial Biomarkers of Response to Experimental Treatment in Early-Phase Clinical Trials in Spondylarthritis

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**Objective.** To identify biomarkers for effective treatment in early-phase clinical trials of spondylarthritis (SpA), by analyzing which synovial features can be reliably identified in patients with SpA.

**Methods.** Synovial biopsies were performed at weeks 0 and 12 in 20 SpA patients treated with infliximab, 20 treated with etanercept, and 12 who were not treated. Primary clinical outcome measures were patient and physician global assessment of disease activity. Extensive histologic evaluation included assessment of lining layer hyperplasia, vascularity, markers of cellular infiltration, and metalloproteinases (MMPs) in the lining and sublining layers.

**Results.** Changes in levels of CD163 (resident tissue macrophages) in the lining, and CD163, MMP-3,

and myeloid-related protein 14 (MRP-14; infiltrating myeloid cells) in the sublining correlated significantly with changes in the primary clinical outcomes. Comparison between responders (n = 35) and nonresponders (n = 17) showed differences in the degree of change in the levels of CD163 in the lining and CD163, MMP-3, and CD3 in the sublining, whereas trends in change in the levels of MRP-8 and MRP-14 in the lining and sublining were similar in the 2 groups. Accordingly, the highest differences in standardized response means (SRMs) between the 2 groups were found for CD163 in the lining, MMP-3, CD163, CD3, and MRP-8 in the sublining, and the level of polymorphonuclear cells (PMNs). When comparing treated and untreated patients, high differences in SRMs were again found for CD163 in the lining, MMP-3, CD163, and MRP-8 in the sublining, and PMNs. These parameters performed prognostically as well as the erythrocyte sedimentation rate and better than the C-reactive protein level. Class prediction analysis yielded a 90% correct prediction using 8 synovial parameters, as follows: lining and sublining CD163, MRP-8, and MRP-14, sublining MMP-3, and PMNs. In validation analyses with independent samples, effective treatment was correctly predicted in 24 of 30 SpA patients and in 2 of 2 placebo-treated patients.

**Conclusion.** Changes in synovial macrophage subsets, PMN levels, and MMP-3 expression reflect response to treatment in SpA. The ability of these parameters to correctly identify effective therapy makes them interesting biomarkers for use in early-phase clinical trials in SpA.

The development of highly effective treatments such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) blockade for the

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Supported by the European Community's FP6 funding. Dr. De Rycke's work was supported by a grant from the Vlaams instituut voor de bevordering van het wetenschappelijk-technologisch onderzoek in de industrie (IWT/SB/11127). Dr. Baeten is a Senior Clinical Investigator of the Fund for Scientific Research-Flanders (FWO-Vlaanderen).

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Submitted for publication June 16, 2005; accepted in revised form March 15, 2006.

most common forms of chronic autoimmune arthritis, including rheumatoid arthritis (RA) (1,2) and the spondylarthritides (SpA) (3–6), makes it increasingly difficult on clinical and ethical grounds to perform placebo-controlled long-term studies with these newer drugs. However, the incomplete response or nonresponse to TNF $\alpha$  blockade in a subset of patients (7), the occasional occurrence of serious side effects (8), and the lack of complete and long-lasting remission in most patients (9) emphasize the need to explore new treatment strategies. It is therefore important to develop new approaches that allow proof-of-concept in early-phase clinical trials of limited size and treatment duration.

Because the synovial membrane is the primary target of the autoimmune inflammation in these diseases, synovial tissue analysis is a straightforward approach for addressing this new challenge. In RA, a large number of studies have indicated that effective treatment leads to marked down-regulation of synovial inflammation, including infiltration with mononuclear cells, expression of cytokines and chemokines, endothelial activation, and expression of adhesion molecules and matrix metalloproteinases (MMPs) (10). A 2-week prospective study of prednisolone treatment showed that, of the variety of synovial features that were down-regulated by effective treatment, the reduction in sublining macrophages was the largest change (11). A followup study analyzing 111 RA patients from trials of 10 different therapeutic regimens confirmed that changes in sublining macrophage levels consistently reflected changes in the Disease Activity Score (DAS) (12), thereby identifying synovial sublining macrophages as a useful biomarker for use in RA clinical trials (13).

Recently, extensive analysis of synovial immunopathology has also been performed in SpA. Findings of major importance were that some synovial features clearly differ between SpA and RA (14–16), that disease-specific features such as marked hypervascularity and CD163+ resident tissue macrophage infiltration may be closely related to the disease pathogenesis (17–21), and that the synovial immunopathology is largely similar in the phenotypically different SpA subtypes, including psoriatic arthritis (PsA) (22,23). Of interest, a cross-sectional analysis of almost 100 SpA synovial biopsy specimens indicated that specific synovial features such as infiltration with CD163+ macrophages and polymorphonuclear cells (PMNs) reflected global disease activity (23). Moreover, several studies using sequential synovial biopsy analysis demonstrated a profound immunomodulation of synovitis in SpA (24–28) and in the PsA subset (29–34) with effective

treatment, including down-regulation of inflammatory infiltration with various monocyte subsets and T lymphocytes, of hypervascularity and endothelial activation, and of the expression of effector molecules such as MMPs.

Given the previous identification of synovial biomarkers in RA, the differences in synovial immunopathology between SpA and RA, the correlation between histologic features and global disease activity in SpA observed in a cross-sectional study, and the modulation of synovial pathology by effective treatment in SpA, the present study was undertaken to identify synovial biomarkers of treatment response that can be used to discriminate between effective and ineffective treatment in early-phase clinical trials in SpA.

## PATIENTS AND METHODS

**Patients.** This 12-week study included 52 patients who had SpA that fulfilled the European Spondylarthropathy Study Group criteria (35) and had active peripheral disease with at least 1 swollen knee joint at baseline. The demographic features of the patients are shown in Table 1. Twenty of these patients were treated with infliximab (5 mg/kg intravenously) at weeks 0, 2, and 6, as described previously (26). Another 20 were treated with etanercept (25 mg twice per week subcutaneously) (28). None of these 40 patients was receiving concomitant treatment with a disease-modifying antirheumatic drug (DMARD). Of the remaining 12 patients, 4 were not treated during the study period and 8 were receiving stable doses of sulfasalazine, which had been initiated at least 3 months before enrollment, but had persistent active disease. This latter group (referred to hereinafter as the control group) consisted of 6 patients who had biopsies performed before the time TNF $\alpha$  blockade treatment became available for SpA but were subsequently included in anti-TNF trials in SpA, 3 patients who were screened to be included in an anti-TNF study but were excluded due to positive findings on a tuberculosis skin test, and 3 patients who underwent biopsies during the placebo phase of a double-blind, placebo-controlled trial of infliximab in SpA (26). Their baseline clinical features (Table 2) and histologic characteristics (data not shown) were comparable with those of the infliximab and etanercept groups. Because we previously showed that there were no major differences in synovial histopathology (22,23) or in clinical or histologic response to TNF blockade treatment (4,25,28) between SpA subgroups, we considered ankylosing spondylitis (AS), PsA, and undifferentiated SpA collectively as SpA. The study was approved by the Ethical Committee of Ghent University Hospital, and all patients provided written informed consent prior to enrollment.

**Clinical outcomes.** Because there are no specific disease activity indices that have been validated across all SpA subgroups, the primary clinical outcome measures used in this study were patient and physician global assessment of disease activity on a 100-mm visual analog scale (VAS patient and VAS physician, respectively) (4). Secondary clinical outcome measures included swollen joint count and tender joint count,

**Table 1.** Demographic features of the 52 patients with SpA, by treatment group\*

	Infliximab (n = 20)	Etanercept (n = 20)	Control (n = 12)†
Age, median (range) years	49 (38–66)	38 (20–71)	43 (20–66)
Male/female	16/4	13/7	11/1
Disease duration, years	18 (1–43)	10 (1–41)	8 (2–34)
Ankylosing spondylitis	10	5	4
Psoriatic arthritis	8	6	2
Undifferentiated SpA	2	9	6
Stable DMARD treatment	0	0	8

\* Except where indicated otherwise, values are the number of patients. SpA = spondylarthritis; DMARD = disease-modifying antirheumatic drug.

† Four patients were receiving no treatment and 8 were receiving sulfasalazine that had been initiated at least 3 months prior to study enrollment, but had persistent active disease.

as well as serum C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) as biologic parameters of inflammation. In an additional subanalysis of AS patients with axial disease, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (36) was used, and, although it has not been validated in SpA, we used the DAS in patients with pronounced polyarticular peripheral disease. For the identification of synovial features that can be used to discriminate between a good clinical response and a moderate response or lack of response, clinical responders were defined as patients with an improvement of  $\geq 50\%$  in one or both primary clinical outcome measures (VAS patient or VAS physician).

**Synovial histology.** Synovial biopsies (16 per patient) were obtained by needle arthroscopy of the knee at week 0 and week 12, as described previously (24–28). As a criterion for enrollment (in order to avoid biases due to local disease activity or treatment with DMARDs), patients had to have clinical effusion of the knee joint at baseline (15,22). The same knee joint was biopsied at baseline and at week 12, independent of the clinical response and the persistence or resolution of the knee effusion. Eight biopsy specimens were stored in formaldehyde and embedded in paraffin and 8 were snap-frozen and mounted in Jung tissue freezing medium (Leica Instruments, Nussloch, Germany) and used for immunohisto-

chemistry analysis. The procedure for histologic and immunohistochemical analysis of the different markers has been validated and extensively described previously (24–28). Briefly, paraffin-embedded biopsy specimens were stained with hematoxylin and eosin for histologic analysis, including determination of mean synovial lining layer thickness (scored on a 1–3 scale, in which 1 = mean 1–2 cell layers, 2 = mean 3–5 cell layers, and 3 = mean  $>5$  cell layers), vascularity of the sublining layer, global cellular infiltration of the sublining layer, and presence of lymphoid aggregates, plasma cells, and PMNs.

**Immunohistochemistry.** Frozen sections of the synovial biopsy samples were stained with the following monoclonal antibodies: 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-CD20 (B cells) (clone L26; Dako), anti-CD68 (pan-macrophage marker) (clone EBM11; Dako), anti-CD138 (plasma cells) (clone CBL455; Chemicon, Temecula, CA), anti-CD146 (endothelial cells) (clone P1H12; Chemicon), anti-CD163 (scavenger receptor expressed on resident tissue macrophages) (clone Ber-MAC3; Dako), anti-MMP-3 (stromelysin 1) (clone SL-1 IIIC4; Oncogene, San Diego, CA), and anti-MMP-9 (gelatinase B, type IV collagenase, IgG1) (clone 36020.111;

**Table 2.** Clinical response to treatment in SpA patients treated for 12 weeks with infliximab or etanercept, and in control patients\*

	Infliximab (n = 20)		Etanercept (n = 20)		Control (n = 12)	
	Week 0	Week 12	Week 0	Week 12	Week 0	Week 12
Patient global assessment, 100-mm VAS	69 (17–100)	15 (0–73)†	73 (18–100)	14 (0–69)†	79 (24–90)	67 (47–98)
Physician global assessment, 100-mm VAS	64 (35–89)	15 (8–75)†	54 (36–89)	15 (1–59)†	43 (20–94)	62 (27–89)
Patient pain assessment, 100-mm VAS	69 (14–100)	16.5 (1–86)†	69 (17–99)	12 (0–80)†	68.5 (9–90)	73 (37–97)†
SJC	7 (0–24)	1 (0–7)†	3 (1–19)	1 (0–10)†	2 (1–11)	2 (1–24)
TJC	10 (1–20)	0 (0–6)†	5 (0–23)	1 (0–13)†	2 (0–6)	3 (0–20)
CRP, mg/dl	2.3 (1.0–7.4)	0.3 (0–7.9)†	1.0 (0–15.0)	0.3 (0–3.4)†	3.1 (0.2–15.8)	1.8 (0.1–7.4)
ESR, mm/hour	24 (11–101)	6 (1–34)†	16 (1–86)	8 (1–39)†	21 (5–107)	17 (4–101)

\* Values are the median (range). SpA = spondylarthritis; VAS = visual analog scale; SJC = swollen joint count; TJC = tender joint count; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

†  $P < 0.05$  versus week 0.

R&D Systems, Abingdon, UK). For myeloid-related protein 8 (MRP-8) and MRP-14 (infiltrating monocyte/macrophages in an early stage of differentiation), monospecific affinity-purified rabbit antisera were used (27,28). After incubation with the primary antibody, sections were sequentially incubated with a biotinylated second antibody, a streptavidin-horseradish peroxidase link, and aminoethylcarbazole substrate as chromogen. Parallel sections were incubated with irrelevant isotype- and concentration-matched antibodies as negative controls.

**Semiquantitative scoring.** Sections were coded and analyzed by 2 independent observers (EK and DB), who were blinded with regard to the clinical data, treatment protocol, and time of biopsy sampling. The analysis included all areas of the 8 biopsy specimens, and a global score was given for each parameter, using a semiquantitative 4-point scale in which 0 represented the lowest and 3 the highest level of expression (24–28,37). Since some histologic markers are more abundant than others, the scoring system was calibrated for each marker separately by examining a representative number of samples. For CD68, CD163, MMP-3, MMP-9, MRP-8, and MRP-14, synovial lining staining and sublining staining were scored separately. In the event of discordant scores (which differed by a maximum of 1 point), the mean of the 2 scores was used.

**Statistical analysis.** The changes in clinical and histologic scores were expressed as the percentage change compared with baseline values. Correlations between these changes were assessed with Pearson's correlation test. Student's *t*-test was used for comparison between responders and nonresponders. *P* values less than 0.05 were considered significant. Standardized response means (SRMs) of the clinical and histologic scores were calculated as the mean change in a score divided by the standard deviation of the change in that score over the 12-week study period. An SRM of >0.8 is considered high, indicating a good ability to detect changes over time. An SRM of 0.5 is considered as indicating moderate potential to detect changes, and an SRM of 0.2 as indicating low potential (13). Finally, class prediction of individual patients, to discriminate between effective and placebo treatment, was performed, using PAM (Predictive Analysis of Microarray) software (38).

## RESULTS

**Clinical response to treatment.** The clinical responses to treatment in the different subgroups (infliximab treatment, etanercept treatment, and control) are shown in Table 2. A significant and pronounced amelioration of all disease activity parameters (patient global assessment of disease activity, physician global assessment of disease activity, patient assessment of pain, swollen and tender joint count, CRP, and ESR) over the 12-week period was observed in both groups receiving TNF $\alpha$  blockade treatment. In contrast, there was no amelioration of these parameters in the control group,

and there was in fact a significant deterioration in patient assessment of pain.

**Correlations between clinical response and histopathologic changes.** We previously demonstrated that specific synovial features reflected global disease activity in SpA, in a cross-sectional study (23). Therefore, in this prospective, longitudinal study of 52 patients, we analyzed whether changes in synovial parameters correlated with changes in disease activity parameters over a 12-week period across different treatment groups. As shown in Table 3, changes in lining and sublining CD163 expression and in sublining MMP-3 expression correlated significantly with changes in both the VAS patient ( $P = 0.045$ ,  $P = 0.036$ , and  $P = 0.023$ , respectively) and the VAS physician ( $P = 0.016$ ,  $P = 0.025$ , and  $P = 0.009$ , respectively). Additionally, changes in sublining MRP-14 expression correlated with changes in the VAS physician ( $P = 0.020$ ). These 4 synovial parameters also correlated significantly with changes in the swollen joint count ( $P = 0.001$ ,  $P < 0.001$ ,  $P < 0.001$ , and  $P = 0.014$ , respectively), as was also the case for sublining MRP-8 expression ( $P = 0.040$ ), PMN level ( $P = 0.016$ ), and global degree of inflammatory infiltration ( $P = 0.015$ ). Overall, the correlations with swollen joint count were stronger than with VAS patient and VAS physician (Table 3). Of interest, none of the 24 synovial features investigated reflected changes in the CRP or ESR, with the exception of CD20+ B lymphocytes, which correlated inversely with changes in both the CRP ( $P = 0.016$ ) and the ESR ( $P = 0.024$ ).

These data indicate that synovial features in SpA not only reflect global disease activity cross-sectionally, but also mirror changes in disease activity occurring over time. This was further confirmed in an additional subanalysis of patients with axial disease ( $n = 16$ ), in whom changes in the BASDAI correlated significantly with changes in levels of PMN ( $P = 0.028$ ), CD163 ( $P = 0.008$ ), MRP-8 ( $P = 0.016$ ), and MRP-14 ( $P = 0.009$ ) in the lining, and levels of CD163 ( $P = 0.010$ ) and MMP-3 ( $P = 0.001$ ) in the sublining. Changes in the DAS in patients with pronounced peripheral disease (mean 6 swollen joints;  $n = 28$ ) correlated with changes in CD163 in the lining ( $P = 0.044$ ) and correlated inversely with changes in CD20 ( $P = 0.007$ ).

**Comparison of clinical responders and nonresponders.** Focusing on patient and physician assessments of global disease activity as the primary clinical outcome parameters, we next assessed which synovial features changed significantly in responders ( $n = 35$ ) compared with nonresponders ( $n = 17$ ). As shown in

**Table 3.** Correlation coefficients between changes in synovial features and changes in clinical parameters in 52 SpA patients over a study period of 12 weeks\*

Synovial feature	Patient global assessment	Physician global assessment	SJC	CRP	ESR
Lining layer thickness	-	-	-	-	-
Vascularity	-	-	-	-	-
Global infiltration	-	-	0.340	-	-
Lymphoid aggregates	-	-	-	-	-
Plasma cells	-	-	-	-	-
PMNs	-	-	0.337	-	-
CD3	-	-	-	-	-
CD4	-	-	-	-	-
CD8	-	-	-	-	-
CD20	-	-	-	-0.335	-0.317
CD138	-	-	-	-	-
CD146	-	-	-	-	-
Lining CD68	-	-	-	-	-
Sublining CD68	-	-	-	-	-
Lining CD163	0.265	0.353	0.469	-	-
Sublining CD163	0.309	0.331	0.524	-	-
Lining MRP-8	-	-	-	-	-
Sublining MRP-8	-	-	0.289	-	-
Lining MRP-14	-	-	-	-	-
Sublining MRP-14	-	0.341	0.341	-	-
Lining MMP-3	-	-	-	-	-
Sublining MMP-3	0.334	0.381	0.540	-	-
Lining MMP-9	-	-	-	-	-
Sublining MMP-9	-	-	-	-	-

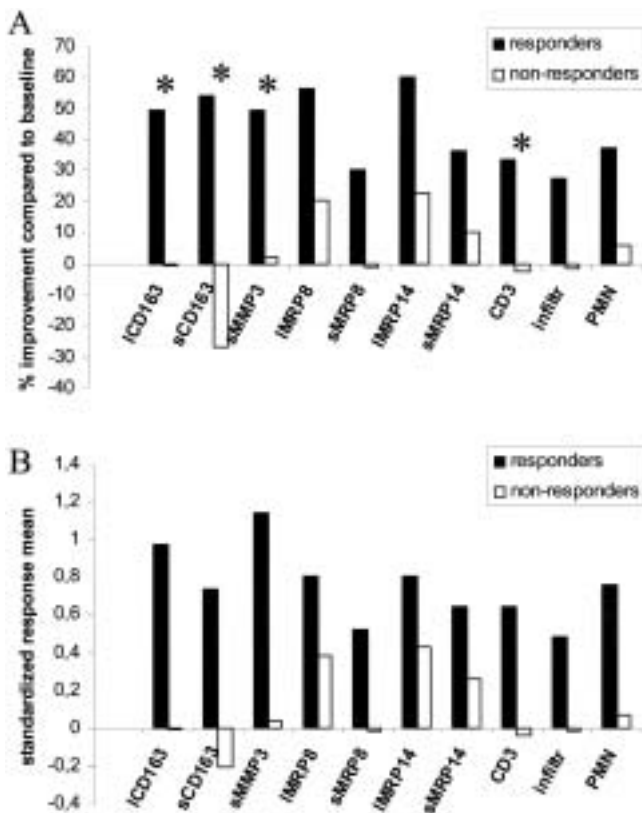
\* Only correlations with *P* values less than 0.05 are shown. PMNs = polymorphonuclear cells; MRP-8 = myeloid-related protein 8; MMP-3 = matrix metalloproteinase 3 (see Table 2 for other definitions).

Figure 1A, changes in lining and sublining CD163 expression (*P* = 0.008 and *P* = 0.010, respectively) and in sublining MMP-3 expression (*P* = 0.003) differed the most significantly between responders and nonresponders. Changes in CD3+ lymphocytes were also significantly different between responders and nonresponders (*P* = 0.040), with a similar trend for lining and sublining MRP-8 expression (*P* = 0.072 and *P* = 0.074, respectively) as well as lining and sublining MRP-14 expression (*P* = 0.095 and *P* = 0.095, respectively). Differences in the global degree of inflammatory infiltration and PMN levels were not significant in this comparison.

In order to assess whether these parameters also could be used to detect changes that occurred during the 12-week study period, we next calculated their SRMs in both the responder and the nonresponder groups. As noted above, an SRM of >0.8 indicates a good ability to detect changes over time, and an SRM between 0.5 and 0.8 indicates moderate potential to detect changes (13). As shown in Figure 1B, the highest differences in SRM between the responder and nonresponder groups were found for sublining MMP-3 (1.11), as well as for lining and sublining CD163 (0.99 and 0.94, respectively). Mod-

erately high differences in SRM were found for PMNs (0.69), CD3 (0.67), and sublining MRP-8 (0.054). Lining MRP-8 and lining and sublining MRP-14 had fairly high SRMs in the responder group (0.80, 0.80, and 0.064, respectively), but also in the nonresponder group (0.38, 0.43, and 0.26, respectively). Taken together, these data indicate that changes in sublining MMP-3 expression and lining and sublining CD163 expression are the best histologic markers for good clinical response.

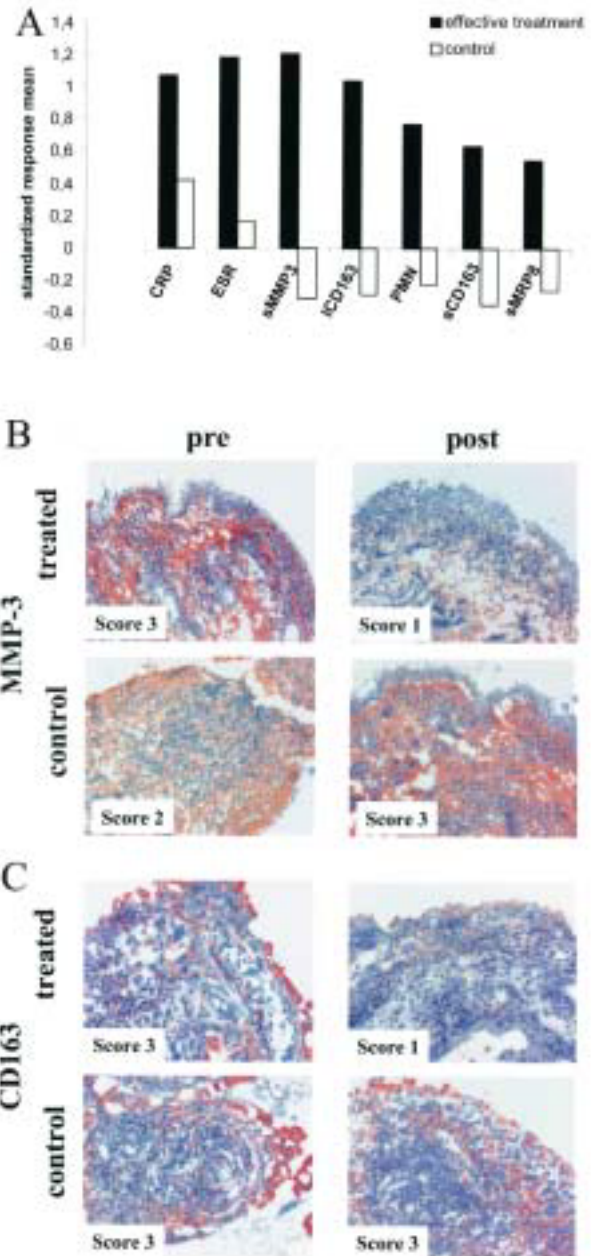
**Comparison between effective and ineffective treatment.** Because the primary aim of this study was to identify histologic markers that were able to discriminate reliably between effective and ineffective treatment rather than merely reflecting clinical response, we next analyzed the SRM in the cohort receiving effective treatment with either infliximab or etanercept (n = 40) and in the control group (n = 12), independently of the individual clinical response. As shown in Figure 2A, high differences in the SRM between these 2 groups were found for sublining MMP-3 expression (1.59), lining CD163 expression (1.33), PMN levels (1.00), sublining CD163 expression (0.99), and sublining MRP-8 expression (0.82). Of major interest, these histologic features performed as well as the ESR (1.02) and better than the



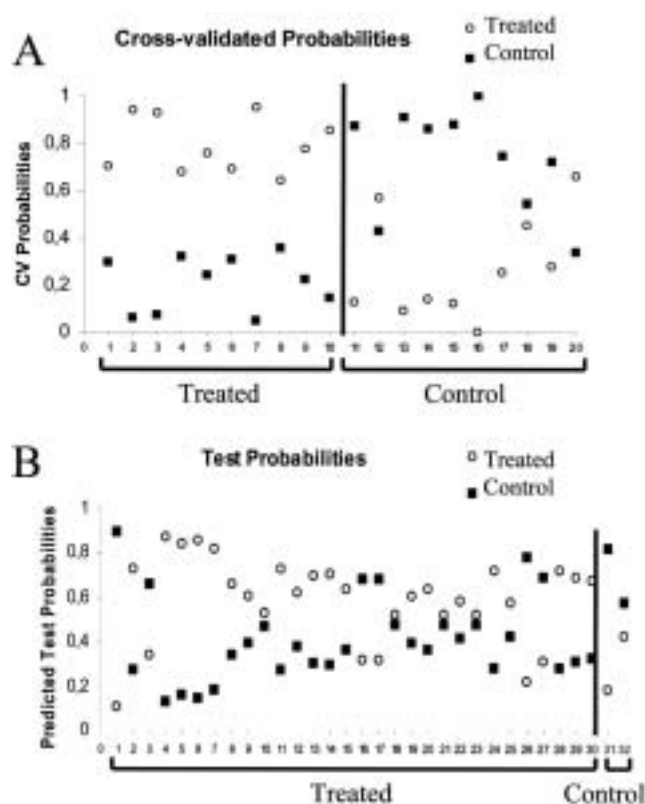
**Figure 1.** Comparison of responders ( $n = 35$ ), defined as patients with improvement of  $\geq 50\%$  in either the patient or the physician global assessment of disease activity, versus nonresponders ( $n = 17$ ). **A**, Mean percent change in the histologic parameters that showed a trend toward a difference ( $P < 0.1$ ) between the 2 groups. \* =  $P < 0.05$ . **B**, Standardized response means, defined as the mean change divided by the standard deviation of this change, for the same parameters. I = lining; s = sublining; MMP-3 = matrix metalloproteinase 3; MRP-8 = myeloid-related protein 8; infiltr = global inflammatory infiltration; PMN = polymorphonuclear cells.

CRP level (0.65), indicating that these synovial features have a similar or superior ability to detect changes in patients receiving effective treatment, compared with the classic biologic parameters. As illustrated for MMP-3 (Figure 2B) and CD163 (Figure 2C), these parameters also performed better than the global degree of synovial inflammatory infiltration. Although the approach used in this study did not allow assessment of the relative changes in specific cell populations compared with global cellularity, the data indicate that these markers are more sensitive to treatment-induced change than is global inflammatory infiltration.

**Class prediction analysis.** To confirm the ability of synovial tissue analysis to discriminate reliably between the effectively treated patients and the control



**Figure 2.** **A**, Comparison of the standardized response mean (SRM) between the patients receiving effective treatment and the control patients. Only those histologic parameters for which the difference in SRM between the 2 groups was  $\geq 0.8$ , indicating good ability to detect changes over the 12-week study period, are shown. For comparison, the SRMs for serum C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) are also shown. **B** and **C**, MMP-3 expression (**B**) and CD163 expression (**C**) in the treated and control groups at the beginning (pre) and end (post) of the 12-week period, illustrating that these changes were more pronounced than the change in global cellular infiltration as determined with a semiquantitative scoring method (score indicated for each sample) (original magnification  $\times 320$ ). See Figure 1 for other definitions.



**Figure 3.** Class prediction analysis using changes in synovial features to discriminate between patients receiving effective therapy and controls. **A**, Samples from 10 treated patients and 10 control patients were used to set up the prediction model. Cross-validation (CV) of this data set indicated that all samples from treated patients were correctly classified with high probability, whereas 8 of 10 samples from control patients were correctly classified. **B**, Application of the model to the 32 samples in the test set, showing that 24 of 30 samples from treated patients and both samples from control patients were correctly classified.

group, we performed a class prediction analysis. With data from 10 treated and 10 control patients randomly chosen from both cohorts, the model identified the following 8 synovial parameters as the best discriminators between effective treatment and control: sublining MMP-3 expression, sublining MRP-14 expression, lining MRP-8 expression, lining CD163 expression, PMNs, sublining CD163, lining MRP-14 expression, and sublining MRP-8 expression. Using these 8 histologic parameters, the model correctly classified all 10 samples from treated patients and 8 of 10 from control patients (Figure 3A). When the model was subsequently applied to the 32 independent test samples, the class prediction algorithm correctly predicted effective or ineffective

treatment in 24 of 30 samples and in 2 of 2 samples, respectively (Figure 3B).

## DISCUSSION

As part of the ongoing search for sensitive and robust biomarkers that can be used in early-phase clinical trials, we undertook the present study to assess which synovial features reflect changes in global disease activity in SpA. We previously demonstrated in a cross-sectional study that resident tissue macrophages, defined by the expression of the group B scavenger receptor CD163, PMNs, and lining layer thickness, were correlated with global disease activity (23). Considering that these synovial features also decrease upon treatment with infliximab and etanercept (26,28), a first important finding of the present study is that the change in synovial CD163+ macrophages correlated with changes over time in disease activity in these patients. This was also the case for MRPs (as markers for infiltrating monocytes) and MMP-3, which were not included in our original cross-sectional study (23) but have been demonstrated in independent studies to reflect global disease activity and peripheral joint disease, respectively, in SpA (25,27) and to decrease rapidly and profoundly upon institution of TNF $\alpha$  blockade therapy (25,27,28). Changes in these synovial parameters and in PMN levels were also significantly more pronounced in clinical responders than in nonresponders and high SRMs for these parameters were observed, reflecting, respectively, their specificity and sensitivity to detect changes in global disease activity.

Taken together, the above findings indicate that changes in global disease activity are accompanied by a series of events in the peripheral joint: inhibition of the influx of new inflammatory monocytes and PMNs from the peripheral circulation, reduction of the resident synovial tissue macrophage population, and down-modulation of inflammation mediators, such as MMP-3, which are at least partially derived from synovial macrophages. These findings are consistent with our recent suggestion that innate immune cells, such as macrophages and PMNs, are the primary driving forces of peripheral joint inflammation in SpA (21).

The mere correlation between changes in synovial features and global disease activity is interesting from a pathophysiologic point of view but is of limited value for clinical application if it does not allow for prediction of clinical efficacy, but only reflects a current condition that is already known. Therefore, the finding that the same synovial features showed highly different

SRMs between SpA patients receiving effective treatment and control patients, independent of the clinical response in individuals, was a second major result of this study. Emphasizing their potential role as biomarkers, they performed as well as the ESR and even better than global cellular infiltration or the CRP level, which was recently proposed to be one of the best predictors of major clinical response to TNF $\alpha$  blockade treatment in AS (39). A class prediction analysis confirmed that assessment of synovial macrophage, PMN, and MMP-3 levels was sufficient to identify reliably effective treatment, even in relatively small cohorts of SpA patients.

Whereas the present study analyzed changes over 12 weeks, we previously demonstrated that levels of features such as PMNs, MRPs, and MMP-3 were also decreased as early as 1 week after initiation of infliximab treatment (24–27). Similarly, studies in RA and PsA have provided evidence of synovial changes occurring as early as 48 hours after initiation of infliximab treatment (32,40), indicating that the value of the proposed synovial biomarkers in SpA should be further tested at earlier time points (2–4 weeks) in future studies. The robust discrimination between the effectively treated cohort and the control group, the good performance compared with classic measurements such as CRP, and the early onset of the synovial changes indicate that synovial macrophages, PMNs, and MMP-3 are useful biomarkers for response to treatment in SpA.

Of interest, other synovial features that were tested did not exhibit the high sensitivity and specificity found for the parameters discussed above. Lining layer hyperplasia, which correlated with disease activity in our cross-sectional study (23), was not identified in the present analysis. Similarly, despite the fact that marked hypervascularity is one of the main features of SpA synovitis (15–18), the decrease in disease activity was not correlated with changes in vascularity. This does not contradict findings of previous studies demonstrating an effect of TNF $\alpha$  blockade on these structural features in SpA, but is in accordance with the concept that short-term modulation of synovial inflammation may be associated with long-term modulation of structural features (24,26,28,31). Alternatively, structural remodeling could be independent of the effect on inflammation, as was recently demonstrated with TNF $\alpha$  blockade in RA (41).

Also, the changes in the different synovial lymphocyte subsets did not appear to reflect clinical improvement in SpA. Although levels of synovial CD3+ lymphocytes have been found to decrease upon initiation of TNF $\alpha$  blockade treatment (24,26,28), the fact that these cells have impaired cytokine production,

which is restored after effective treatment, strongly suggests that they are bystanders rather than drivers of synovial inflammation in SpA (42–45). Accordingly, the decrease in T lymphocytes was more pronounced in responders than in nonresponders but did not contribute to the discrimination between effective treatment and placebo. Even more striking, levels of B lymphocytes and plasma cells have been found to remain stable or even increase in SpA synovium after TNF $\alpha$  blockade treatment, despite major clinical and histologic improvement (24,26,28). In accordance with this, the present study demonstrated that, if anything, there was a correlation between clinical improvement and an increase, rather than a decrease, of synovial CD20+ B lymphocytes.

A number of caveats should be considered when interpreting the present data. First, in contrast with RA, for which the DAS is a well-validated and globally accepted clinical measure of disease activity (13), there is no such equivalent for assessing SpA as a whole. Several clinical measurements have been developed for SpA subtypes such as AS or PsA, but these are not globally applicable to other SpA subtypes. Moreover, strictly speaking, these outcome measures are themselves surrogate markers rather than genuine clinical end points. Therefore, we used patient and physician global assessments of disease activity as primary clinical outcome measures (4), but whether changes in levels of synovial macrophages, PMNs, and MMP-3 also reflect specific disease activity parameters in AS and PsA remains to be further investigated.

Second, we used semiquantitative scoring to assess changes in synovial histopathology. Although this technique is robust and yields essentially similar results to those obtained with manual counting or digital image analysis, it may have a lower sensitivity to change (46–48). The use of one of these quantitative techniques could further improve the value of the proposed synovial biomarkers.

Third, we previously were not able to detect specific differences in synovial histopathology between SpA subtypes (22,23), and all subtypes appeared to have a similar clinical and histologic response to TNF $\alpha$  blockade treatment (4,26,28). In addition, in the present study we performed a subanalysis of the infliximab and the etanercept cohorts and found that the same synovial features are good discriminators between each of these 2 groups and the control group (data not shown). Whereas these data further confirm that the global synovial response to treatment is largely similar with both TNF $\alpha$  blockers, the size of the cohort does not allow us to formally exclude potential minor differences, and fur-

ther research into more specific molecular mechanisms, such as apoptosis, is certainly warranted. The aim of the present study, however, was to find synovial markers of response to treatment that can be used across different treatment regimens, rather than to identify specific mechanisms of action related to one single drug.

Although we have shown that in RA, synovial sublining macrophages were useful biomarkers across a wide variety of treatment regimens (13), it remains to be demonstrated that this is also the case in SpA and that the observed changes in fact indicate global clinical improvement rather than a specific response to TNF $\alpha$  blockade. This may be particularly relevant in SpA because, in contrast with TNF $\alpha$  blockade agents, drugs such as sulfasalazine have been demonstrated to act differentially on peripheral and axial disease (49,50). Therefore, biomarkers of axial response should be developed along with the synovial biomarkers, to ascertain the global efficacy of experimental treatments in SpA. It should be emphasized that the ultimate aim of the development of these biomarkers is to provide a large amount of useful biologic data for short-term, early-phase clinical trials of small patient cohorts, and that this setting is completely different from the development of surrogate markers for clinically relevant outcomes in phase III and IV trials or in clinical practice.

In summary, the results of the present study indicate that changes in expression of synovial macrophage subsets, PMNs, and MMP-3 reflect response to treatment in SpA. Their ability to correctly discriminate between effective and ineffective treatment in small patient cohorts makes them interesting biomarkers to facilitate conclusive early-phase clinical trials in SpA. Further studies are needed to confirm their value as biomarkers at early time points across different therapeutic regimens and to combine synovial assessment with predictors of axial response to treatment in SpA.

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