Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis

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Background: Previous work identified synovial sublining macrophage numbers as a potential biomarker for clinical efficacy in rheumatoid arthritis.

Objective: To investigate the association between changes in infiltration of synovial macrophages and clinical improvement after antirheumatic treatment.

Methods: 88 patients who participated in various clinical trials were studied. All patients underwent serial arthroscopy before initiation of treatment and after different time intervals. Immunohistochemical and digital image analysis were performed according to standardised procedures to detect changes in CD68+ synovial sublining macrophages in relationship to changes in the 28 joint count Disease Activity Score (DAS28). Statistical analysis was performed using one way analysis of variance, the independent samples t test, linear regression, and the standardised response mean (SRM).

Results: For good, moderate, and non-responders, according to the DAS28 response criteria, there was a significant difference in the change in sublining macrophages (mean (SEM) cells/mm² - 643 (124), −270 (64), and −95 (60), respectively; p < 0.0003). There was a significant correlation between the change in the number of macrophages and the change in DAS28 (Pearson correlation 0.874, p < 0.01). The change in sublining macrophages explained 76% of the variation in the change in DAS28 (p < 0.02). The sensitivity to change of the biomarker was high in patients treated actively (SRM > 0.8), whereas the ability to detect changes in placebo treated patients was weak (SRM < 0.3).

Conclusion: The results suggest that changes in synovial sublining macrophages can be used to predict possible efficacy of antirheumatic treatment.

The recent increase in the development of a variety of new, targeted treatments clearly raises the need for sensitive biomarkers, which could be used for selection during the development process. The acquisition of optimal tissue samples has been greatly enhanced by technological developments in needle arthroscopy. Reliable microscopic quantification of synovial inflammation has been facilitated by advances in computerised image analysis. By these means, sequential synovial biopsy specimens have recently been analysed in several clinical trials evaluating the effects of disease modifying antirheumatic drugs (DMARDs), biological treatments, and targeted small molecules. These studies suggested consistent associations between the rapidity and magnitude of both the clinical and immunohistological responses. No consensus, however, has previously emerged as to the optimal markers in tissues that are representative of disease activity or sensitive to change.

A prior cross-sectional study in 62 patients with rheumatoid arthritis (RA) using stepwise multiple regression analysis showed that scores for local disease activity are particularly associated with the number of macrophages in the synovial sublining as well as the expression of macrophage derived cytokines. Recently, we conducted a randomised trial to try to answer the question of which feature in RA synovial tissue (ST) samples could be used as a biomarker for clinical efficacy in relatively small studies of short duration. Patients received either prednisolone according to the COBRA regimen or placebo for 2 weeks. ST samples were obtained before the start of treatment and at 2 weeks. Twenty four immunohistological markers were investigated in this study. Each of the end points was statistically analysed using an analysis model of covariance. The model fitted included terms for treatment as a fixed effect and the baseline measurement as a covariate. The aim was to assess the treatment difference. This study identified sublining macrophages as the best biomarker associated with the clinical response to corticosteroids.

The utility of CD68+ macrophages in the sublining layer as a candidate biomarker now requires to be tested across discrete interventions and kinetics. The objective of this study was to investigate the changes in this biomarker after different treatments and after different time intervals in relationship to the clinical response to treatment to validate the analysis of synovial macrophages in clinical studies.

Patients and Methods

Patients

ST was obtained by arthroscopy under local anaesthesia at two different times from a clinically active wrist, knee, or ankle joint of each of 88 patients who fulfilled the American College of Rheumatology criteria for RA. Before each arthroscopy the 28 joint count Disease Activity Score

Abbreviations: DAS28, 28 joint count Disease Activity Score; DMARDs, disease modifying antirheumatic drugs; IL, interleukin; LEF, leflunomide; MTX, methotrexate; RA, rheumatoid arthritis; SRM, standardised response mean; ST, synovial tissue; TNF, tumour necrosis factor

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Eighty-eight patients were included and analysed in this study. Table 1 shows their baseline disease activity and treatment characteristics. All patients had active disease at baseline as measured by the DAS28 (mean (SEM) 6.02 (0.11)). On average, there were no differences in age, sex, and disease duration between the different subgroups; for further demographic and clinical characteristics we refer to Table 1.  

**Table 1** Treatment characteristics and baseline disease activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Active/control group</th>
<th>Number of patients</th>
<th>Interval (days)</th>
<th>DAS28 baseline Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>Active</td>
<td>15</td>
<td>112</td>
<td>6.15 (4.50–7.83)</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Active</td>
<td>15</td>
<td>112</td>
<td>6.05 (4.38–7.31)</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Active</td>
<td>10</td>
<td>14</td>
<td>6.26 (4.58–7.98)</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Active</td>
<td>20</td>
<td>27</td>
<td>6.07 (4.14–8.21)</td>
</tr>
<tr>
<td>CCR1 antagonist</td>
<td>Active</td>
<td>10</td>
<td>14</td>
<td>5.84 (4.31–6.95)</td>
</tr>
<tr>
<td>Stable MTX</td>
<td>Control</td>
<td>6</td>
<td>2</td>
<td>5.97 (4.22–8.21)</td>
</tr>
<tr>
<td>Stable DMARD</td>
<td>Control</td>
<td>12</td>
<td>14</td>
<td>5.69 (3.43–7.59)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>88</td>
<td>53 (5)</td>
<td>6.02 (0.11)*</td>
</tr>
</tbody>
</table>

*Mean (SEM).
The published data of all subgroups. The 70 patients who started active treatment showed, on average, a significant change in the DAS28 of $-1.37$ (0.14) (mean (SEM)) after treatment ($p<0.001$), whereas the DAS28 remained the same in the 18 control patients (change in DAS28 $-0.13$ (0.12); table 2).

The number of good, moderate, and non-responders was calculated for all patients according to the DAS28 response criteria. In total, 11 patients fulfilled the criteria for good responders (that is, improvement $>1.2$ and DAS28 at end point $<3.2$), 35 patients were moderate responders (that is, improvement $>0.6$ and $<1.2$ and DAS28 at end point $<5.1$, or improvement $>1.2$ and DAS28 at end point $>3.2$), and 42 patients were considered non-responders.

### Changes in sublining macrophages are associated with clinical improvement

Table 2 shows the changes in macrophage numbers in the intimal lining layer and sublining as well as the percentage changes in sublining macrophages compared with baseline, in relationship to changes in the DAS28 for the various trials. There was a significant difference in the mean (SEM) change in sublining macrophages compared with baseline, $-0.23$ (0.10) (fig 2). There were no correlations between the change in intimal macrophages and the change in DAS28 (data not shown). Linear regression analysis, weighted for the number of patients in each substudy, showed that the mean change in sublining macrophages could significantly explain 76% of the variance in the mean change in DAS28 grouped for each substudy ($p<0.02$).

### Changes in sublining macrophages may predict active treatment

To determine the sensitivity to change, SRMs of the changes after treatment were calculated. Figure 3 shows that the SRMs in individual substudies were high for both sublining macrophages and DAS28 after active treatment. When patients from all active treatment substudies were grouped

![Figure 1](https://www.annrheumdis.com)

**Figure 1** Mean values of the change compared with baseline in (A) the number of CD68+ macrophages in the intimal lining layer and (B) the synovial sublining for, respectively, non-responders, moderate responders, and good responders according to the DAS28 response criteria in the total study group.

### Table 2

Mean (SEM) change in DAS28, mean (SEM) change in the number of intimal lining CD68+ macrophages, mean (SEM) change in the number of sublining CD68+ macrophages, and the mean percentage change in sublining CD68+ macrophages compared with baseline for each substudy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in the number of intimal lining CD68+ macrophages Mean (SEM)</th>
<th>Change in the number of sublining CD68+ macrophages Mean (SEM)</th>
<th>Change for sublining CD68+ macrophages Mean %</th>
<th>Change in DAS28 Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone start (10; 14)</td>
<td>$-35$ (46)</td>
<td>$-492$ (89)</td>
<td>$-51$</td>
<td>$-2.15$ (0.40)</td>
</tr>
<tr>
<td>Infliximab (Remicade) (20; 28)</td>
<td>$-100$ (30)</td>
<td>$-275$ (84)</td>
<td>$-47$</td>
<td>$-1.39$ (0.28)</td>
</tr>
<tr>
<td>Leflunomide start (15; 112)</td>
<td>$-28$ (17)</td>
<td>$-286$ (112)</td>
<td>$-38$</td>
<td>$-1.29$ (0.31)</td>
</tr>
<tr>
<td>Methotrexate start (15; 112)</td>
<td>$-44$ (17)</td>
<td>$-292$ (106)</td>
<td>$-41$</td>
<td>$-1.36$ (0.25)</td>
</tr>
<tr>
<td>CCR1 antagonist (10; 14)</td>
<td>$-192$ (81)</td>
<td>$-338$ (131)</td>
<td>$-44$</td>
<td>$-0.72$ (0.27)</td>
</tr>
<tr>
<td>Stable DMARD (12; 14)</td>
<td>$+40$ (38)</td>
<td>$+152$ (107)</td>
<td>$+13$</td>
<td>$-0.23$ (0.10)</td>
</tr>
<tr>
<td>Stable MTX (6; 2)</td>
<td>$-71$ (29)</td>
<td>$+26$ (158)</td>
<td>$+22$</td>
<td>$+0.07$ (0.28)</td>
</tr>
<tr>
<td>Active treatment grouped (70; 53)</td>
<td>$-76$ (17)</td>
<td>$-321$ (46)</td>
<td>$-44$</td>
<td>$-1.37$ (0.14)</td>
</tr>
<tr>
<td>Controls grouped (18; 10)</td>
<td>$-3$ (29)</td>
<td>$+110$ (87)</td>
<td>$+17$</td>
<td>$-0.13$ (0.12)</td>
</tr>
</tbody>
</table>
A biomarker for response to treatment in patients with RA

DAS28 (clinical signs of inflammation). In keeping with this concept, suggesting that macrophage numbers are associated with expression of macrophage derived cytokines (tumour necrosis disease activity and the number of macrophages as well as demonstrated a positive correlation between scores for local efficacy of a new antirheumatic treatment. Of importance, the data indicate the numbers of sublining macrophages may be used to explain clinical outcome. Of interest, the data indicate that the change in the number of sublining macrophages may also be reduced after antirheumatic treatment, depending on the specific mechanism of action and the duration of treatment. Obviously, these specific cell types and their mediators of inflammation also need to be evaluated in studies focusing on the mechanism of action of targeted treatments. The immunohistological variables may correlate with each other to a certain extent. Recently, we conducted a study to provide a greater understanding of the changes in the ST alongside clinical response by using a known clinically effective treatment, prednisolone. The analysis model of covariance showed that clinically effective prednisolone treatment was particularly associated with a marked reduction in macrophage infiltration in the RA ST after 2 weeks of treatment. Comparable results were obtained after infliximab treatment.

As a result of these observations, we investigated whether this biomarker might exhibit similar changes after different therapeutic regimens and after varying lengths of treatment. The results of this study show that this is the case. There is a highly significant correlation between changes in sublining macrophages and clinical improvement, independent of the specific treatments studied. The changes may be observed as
early as 14 days after initiation of effective treatment, but
treatment for more prolonged periods results in a more
pronounced decrease in macrophage infiltration. Patients
who are good, moderate, or non-responders according to the
DAS28 response criteria differ significantly in the changes in
the number of sublining macrophages. Additionally, it is
possible to explain the change in DAS28 based upon the
change in sublining macrophages, which implies a direct
relationship between macrophages and clinical measures of
disease activity.

Moreover, we investigated the sensitivity to change of this
biomarker after active treatment or placebo. According to the
SRM, the sensitivity to change after active treatment is good
for both the DAS28 and sublining macrophages. The SRMs
calculated for changes in DAS28 and sublining macrophages
after placebo treatment suggest that the biological marker
may be less susceptible to placebo effects or expectation bias
than clinical evaluation. This might be explained by the
subjective components included in clinical measures of
disease activity. This notion is supported by a previous
study in an independent patient cohort using semi-quantita-
tive analysis, showing unaltered immunohistological scores
in serial synovial biopsy specimens obtained after placebo
treatment.

In conclusion, the results of this study indicate that
sy novial sublining macrophages might be used as a biomar-
ker for the evaluation of new antirheumatic treatments.
In addition to providing insight into the mechanism of action
of treatment, this approach may help to screen for possible
efficacy.

ACKNOWLEDGEMENTS

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Haringman, Gerlag, Zwinderman, et al
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Ann Rheum Dis 2005 64: 834-838 originally published online December 2, 2004
doi: 10.1136/ard.2004.029751