Synovial Macrophages as a Biomarker of Response to Therapeutic Intervention in Rheumatoid Arthritis: Standardization and Consistency Across Centers

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ABSTRACT. Successive studies from one academic center (Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands) have consistently suggested that synovial tissue expression of sublining macrophages may be a biomarker of clinical response to therapeutic intervention in rheumatoid arthritis (RA) clinical trials. A proof-of-concept, randomized clinical trial was completed at a second academic center (St. Vincent's University Hospital, Dublin, Ireland), and the relationship between the change in disease activity and the change in sublining macrophages in distinct treatment cohorts was determined. The preliminary findings were not conclusive, but appeared to support a role for sublining CD68+ macrophages as a biomarker of clinical response to therapeutic intervention in cohorts of patients with RA. (J Rheumatol 2007;34:620–2)

Key Indexing Terms:
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Sublining macrophages as a marker of response to treatment

It has been proposed, based on considerable evidence, that synovial tissue expression of CD68, a phenotypic marker of macrophages, may be a biomarker of response to therapeutic intervention in rheumatoid arthritis (RA) clinical trials[1]. Initially, a cross-sectional study of 62 patients with RA used stepwise multiple regression analysis to show 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[2]. Subsequently, a randomized trial was designed to answer the question of which feature in RA synovial tissue samples could be used as a biomarker for clinical efficacy in relatively small studies of short duration[3]. Patients received either prednisolone according to the COBRA regimen or placebo for 2 weeks. This study identified sublining macrophages as the best biomarker associated with the clinical response to corticosteroids. Next, the utility of CD68+ macrophages in the sublining layer as a candidate biomarker was tested across discrete interventions and kinetics[4]. Data were derived from randomized clinical trials (RCT) that evaluated 5 active therapeutic compounds, methotrexate[5], leflunomide[5], prednisolone[3], infliximab[6], and a CCR1 antagonist[7], and 3 patient cohorts receiving stable disease-modifying antirheumatic drug (DMARD) therapy combined with placebo[5-7]. The study duration of the RCT ranged between 2 and 112 days, and the number of patients with RA randomized to each treatment group was between 6 and 20. All studies were completed in the same center, using the same clinical outcome measure, the same arthroscopic techniques, and the same methodologies for tissue handling, staining and digital image analysis.

A strong correlation between the mean change in disease activity score (ΔDAS28) and the mean change in the number of CD68+ sublining macrophages was observed (Pearson correlation coefficient 0.874, $p < 0.01$). When patients from all actively treated studies were grouped ($n = 70$), the standardized response mean (SRM), a measure of sensitivity to change, was 1.16 for the change in DAS28 and 0.83 for the change in sublining macrophages, indicating good sensitivity to change for both variables. For the patients from the 2 control groups, the SRM was –0.23 and 0.30, respectively, consistent with the notion that the biomarker is less susceptible to placebo effects or expectation bias than clinical evaluation, which includes subjective measures of disease activity.

Sublining macrophages after placebo or ineffective treatment

In addition to its role as a marker of response to effective treatment, the change in numbers of CD68+ sublining macrophages could also help to distinguish effective from ineffective treatment. In this context, it is important that sublining macrophages do not appear to change after placebo or ineffective treatment. There were no changes in CD68+ sublining macrophages in the 3 patient cohorts receiving stable DMARD therapy combined with placebo[4]. At the OMERACT 8 meeting, new data were presented showing that ineffective treatment with anti-monocyte chemoattractant protein-1 antibodies or an oral C5aR antagonist did not induce any change in sublining macrophages (Wijbrandts CA, *et al*, unpublished observations). Inclusion of these new data in the previous analysis on the utility of CD68+ macrophages in the synovial sublining as a candidate biomarker across discrete interventions and kinetics[4] resulted in a correlation coefficient between the mean change in DAS28 and the mean change in CD68+ sublining macrophages of 0.904 ($p < 0.001$) (Wijbrandts CA, *et al*, unpublished observations). These data confirm and extend previous studies using in part different methodology, but with the same results. Treatment with interleukin 10 produced no measurable therapeutic effect, and no change in synovial tissue morphology, including sublining macrophage infiltration[8].

A subtherapeutic dose of anakinra (30 mg/day) also failed to alter synovial tissue morphology after 24 weeks[9]. A depleting anti-CD4 monoclonal antibody resulted in a reduction in the number of sublining CD4+ lymphocytes, but no therapeutic effect and no change in the number of sublining macrophages[10]. Similarly, 2 independent studies have shown that interferon-ß therapy did not affect the number of sublining macrophages[11,12]. Similar observations were recently published by an independent group, underscoring the consistency of these findings[13]. Taken together, these observations suggest that therapies that fail to reduce the number of synovial sublining macrophages are unlikely to be clinically effective.

Standardization of methodology

Before the use of this biomarker can be generally applied in early phase, proof-of-concept clinical trials, standardization of methodology is critical. Many technical aspects have been standardized by the EULAR Synovitis Study Group over the last decade[1,14]. More recently, strong interobserver agreement was demonstrated for microscopic measurement of synovial inflammation using manual quantitative, semiquantitative, and digital methods of analysis[15]. Good interobserver agreement was demonstrated for all 3 methods of analysis. Using manual methods, intraclass correlation coefficients (ICC) for measurement of CD3+ and CD68+ cell infiltration were 0.73 and 0.73 for quantitative analysis and 0.83 and 0.78 for semiquantitative analysis, respectively. Corresponding ICC of 0.79 and 0.58 were observed for the use of digital image analysis. At each participating center, use of image analysis produced results that correlated strongly and significantly with those obtained using manual measurement.

In a proof-of-concept RCT of cytokine blockade completed in Dublin, DAS28 scores and synovial tissue CD68 expression were calculated at baseline, 4 weeks, and at the final study timepoint (median 32 weeks; Rooney T, *et al*, unpublished observations). In a preliminary analysis, the changes in DAS28 values were correlated with the changes in CD68 staining, producing a correlation coefficient $r = 0.80$. Moreover, a good DAS28 response was associated with the greatest mean change in CD68 staining (–20.3%). A moderate DAS28 response was associated with an intermediate mean change in CD68 staining (–10.8%), and failure to demonstrate a DAS28 response was associated with minimal mean change in CD68 staining (–6.8%). These observations support the proposal that CD68 may be a synovial tissue biomarker of therapeutic response in RA.

Conclusion

The change in the number of CD68+ macrophages in the synovial sublining can assist in screening for possible antirheumatic effects of new compounds in patients with RA. Together with evaluation of the specific effects of a targeted drug at the site of inflammation providing proof of concept (in addition to initial evaluation of safety and clinical efficacy), this biomarker may help to make a go/no go decision in an early stage of drug development. Despite differences in analysis techniques, the consistent relationship between changes in sublining macrophages and efficacy has now been confirmed by several centers.

Future research will need to focus on further standardization of methodology across different centers. The use of emerging technologies like tissue-ELISA, quantitative polymerase chain reaction (Q-PCR), and microarray analysis in measuring therapeutic effects in serial synovial biopsies is another focus of research; data on the value of Q-PCR are expected shortly. Finally, identification of optimal biomark-

ers, including synovial macrophage subpopulations, in conditions other than RA is evoking considerable interest[16]. Studies designed to identify the optimal tissue biomarker in trials of treatment for patients with psoriatic arthritis are under way.

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