Biomarkers of outcome in rheumatoid arthritis

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SYNOVIAL TISSUE BIOMARKERS IN CLINICAL TRIALS

Early, open-label clinical studies demonstrated that the magnitude of the therapeutic response to standard disease-modifying anti-rheumatic drugs (DMARDs) in rheumatoid arthritis (RA) was associated with measurable changes in synovial tissue morphology after treatment. Subsequent open-label studies in RA and other categories of chronic arthritis further highlighted specific effects of treatments such as methotrexate, corticosteroids, and infliximab on mononuclear cell infiltration, and on the expression of pro-inflammatory and matrix-degrading mediators in synovial tissue. These studies provided valuable insights into the pathophysiology of RA, and highlighted mechanisms of disease modulation by established and novel treatment modalities.

In recent years, synovial tissue was evaluated before and after treatment in several randomized clinical trials

(RCTs) of both DMARDs and biologic agents. These studies, some of which were placebo-controlled, substantially increased the validity of earlier observations, and highlighted in particular the consistent relationship between the change in the intensity of sub-lining macrophage infiltration and the magnitude of the clinical response. In particular, one study was designed to identify the optimal IHC biomarker of clinical efficacy in relatively small patient cohort following a short treatment duration. Patients received either prednisolone according to the COBRA regimen or placebo. Synovial biopsies were obtained before initiation of treatment and after 2 weeks. Twenty-four protein markers were evaluated by IHC, and 4 additional mRNA markers by quantitative PCR. Each of the endpoints was statistically analyzed using an analysis model of covariance (ANCOVA). 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. The study confirmed the status of sub-lining layer macrophages as the optimal biomarker of the clinical response to corticosteroids. Subsequently, the merit of using the number of sub-lining macrophages as a candidate biomarker was tested across a range of discrete interventions and kinetics. Eighty-eight patients who participated in various randomized clinical trials were evaluated in the same center, using standardized techniques. The treatments evaluated included methotrexate, leflunomide, prednisolone, infliximab, a specific CCR1 inhibitor, and placebo. All patients had baseline and follow-up biopsies and disease activity scores (DAS) performed. There was a significant correlation between the change in the number of macrophages and the change in DAS28. The change in sub-lining macrophages could explain 76% of the variation in the change in DAS28. The sensitivity to change of the biomarker was high in actively treated patients while the ability to detect changes in placebo treated patients was weak. The close correlation was clearly independent of the mode of action of the individual therapies.

It has also been demonstrated that immunohistologic changes in synovium appear very early after the initiation of treatment, and before the appearance of clinical improvement. Thus, 48 hours after the first infusion of 3 mg/kg infliximab, a stastically significant decrease in synovial tissue macrophage numbers was demonstrated. After 1 month, the most pronounced reduction of macrophage numbers was found in the patients with clinical improvement.

A number of biopsy studies on compounds that were not clinically effective reinforce the proposal that an effect on sub-lining macrophage infiltration may represent a reliable biomarker of a therapeutic response. Thus, treatment with interleukin-10 produced no measurable therapeutic effect, and no change in synovial tissue morphology, including sub-lining macrophage infiltration. A sub-therapeutic dose of anakinra (30 mg/day) also failed to alter synovial tissue morphology after 24 weeks. A depleting anti-CD4 monoclonal antibody resulted in a reduction in the number of sub-lining CD4+ lymphocytes, but no therapeutic effect and no change in the number of sub-lining CD68+ macrophages. Similarly, two independent studies have shown that IFN-beta therapy did not affect the number of sub-lining macrophages. These observations suggest that therapies which fail to reduce the number of sub-lining macrophages are unlikely to be clinically effective.

In conclusion, the accumulated data from several studies suggest that sub-lining macrophages may be reliably used as a surrogate marker for arthritis activity when evaluating novel therapies for RA, and may assist in screening for efficacy and in optimizing dose-ranges. The exciting possibility that synovial biopsy may offer predictive utility beyond currently available clinical parameters also arises.

SYNOVIAL TISSUE ANALYSIS AND PREDICTING JOINT DAMAGE

An early synovial biopsy study attempted to identify predictive indices of outcome in RA, and suggested that the intensity of CD68+ macrophage infiltration at baseline was associated with progressive joint damage. This was supported by a later cross-sectional study. A more recent study of patients with early arthritis demonstrated a good correlation between the proportion of lining layer macrophages at baseline and the appearance of new joint erosions. Lining layer macrophages are more highly activated than sub-lining macrophages, express greater amounts of interleukin-1 and tumor necrosis factor-·, and are thought to migrate into the expanding pannus that participates in the degradation of articular cartilage and sub-chondral bone. Matrix metalloproteinase-1 (MMP-1) gene expression in both the lining layer and sub-lining layers was also strongly associated with the formation of new erosions. In this study, the follow-up period was one year in all patients.

Another recent study evaluated 36 patients with early RA, and demonstrated an association between the number of both sub-lining T cells and FLS, and deterioration in the Larsen radiographic score. The follow-up period ranged between 38 and 72 months (mean, 58 months). Differences in the patient characteristics, the intervals between

follow-up biopsies, and the different methods of determining joint damage may explain the discrepancy between the two studies. Taken together, and considering current concepts of disease pathogenesis, it is possible that the accumulation of critical numbers of macrophages in the lining layer, and of cells expressing RANK-ligand (T cells and FLS) might predict joint damage, but that mediators of matrix degradation (eg, MMPs) may ultimately prove to be superior predictors of damage. It is also noteworthy that methotrexate, leflunomide, prednisolone, and infliximab have been associated with decreased expression of MMPs in synovial tissue.

THE POTENTIAL OF SYNOVIAL TISSUE ANALYSIS IN FUTURE RCTS

The synovial membrane is the target tissue in treatment strategies of RA and other arthropathies. Effective modulation of synovitis is critical when attempting to control symptoms and signs, to prevent joint damage, and to maintain function. In RCTs, the systematic evaluation of changes in synovial tissue after commencing treatment enables identification of an early therapeutic effect, using relatively small numbers of patients. First, direct proof of principle may be shown by molecular analysis of the specific effects of the intervention. Second, changes in biomarkers associated with clinical efficacy independent of the primary mechanism of action may help to screen for potential efficacy. Thus, decisions in phase I/II studies may be accelerated, and dose selection enhanced. Finally, synovial tissue analysis at baseline may identify early predictive markers of a likely therapeutic response, as well as markers of future structural damage. These advances will challenge academic rheumatology to optimize the clinical resources and expertise in both arthroscopy and digital image analysis, and will provide opportunities for future collaboration with the pharmaceutical and biotechnology industries.

RESEARCH AGENDA

Further research will depend on effective international collaboration, and on maintaining validation of both existing and evolving methodologies. The proposed research agenda includes the application of synovial tissue analysis to outcomes in other important arthropathies (spondyloarthropathies, psoriatic arthritis, and osteoarthritis) that may be responsive to innovative therapeutic interventions. Collaborative protocols with other clinical and imaging (MRI) research groups are being developed in an attempt to enhance predictive and response indices in tissue. Finally, a comparison between IHC and emerging technologies (eg, quantitative PCR, micro-array, tissue-based ELISA, proteomics) in measuring therapeutic effects is to be evaluated.

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