OMERACT 7 Special Interest Group

Synovial Tissue Analysis in Clinical Trials

BARRY BRESNIHAN, DOMINIQUE BAETEN, GARY S. FIRESTEIN, OLIVER M. FITZGERALD, DANIELLE M. GERLAG, JASPER J. HARINGMAN, IAIN B. McINNES, RICHARD J. REECE, MALCOLM D. SMITH, ANN-KRISTIN ULFGREN, DOUGLAS J. VEALE, and PAUL PETER TAK

ABSTRACT. Synovial tissue analysis has considerable potential for future randomized controlled trials (RCT). The synovial membrane is the target tissue in treatment strategies of rheumatoid arthritis 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 RCT, the systematic evaluation of changes in synovial tissue after commencing treatment enables identification of an early therapeutic effect, using relatively small numbers of patients. This special interest group is working on establishing the evidence to have this endpoint meet the OMERACT filter criteria. (J Rheumatol 2005;32:2481–4)

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From the Department of Rheumatology, St. Vincent's University Hospital, and The Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland; Department of Rheumatology, Ghent University Hospital, Ghent, Belgium; University of California, San Diego, School of Medicine, La Jolla, CA, USA; Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands; Center for Rheumatic Diseases, University of Glasgow, Glasgow; Academic Unit of Musculoskeletal Medicine, University of Leeds, Leeds, UK; Rheumatology Research Unit, Repatriation General Hospital, and Flinders University, Adelaide, Australia; Karolinska Hospital, Stockholm, Sweden.

B. Bresnihan, MD, FRCP, Professor of Rheumatology, Department of Rheumatology, St. Vincent's University Hospital, and The Conway Institute of Biomolecular and Biomedical Research, University College Dublin; D. Baeten, MD, PhD, Professor of Medicine, Department of Rheumatology, Ghent University Hospital; G.S. Firestein, MD, Professor of Medicine, Division of Rheumatology, Allergy and Immunology, University of California, San Diego, School of Medicine; O. FitzGerald, MD, FRCP, Professor of Rheumatology, Department of Rheumatology, St. Vincent's University Hospital, and The Conway Institute of Biomolecular and Biomedical Research, University College Dublin; D.M. Gerlag, MD, Assistant Professor of Medicine, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam; J.J. Haringman, MD, Research Fellow, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam; I.B. McInnes, FRCP, PhD, Professor of Experimental Medicine, University of Glasgow; R. Reece, MB, FRCP, Consultant Rheumatologist, Chapel Allerton Hospital, Academic Unit of Musculoskeletal Medicine, University of Leeds; M.D. Smith, MD, PhD, Professor of Rheumatology, Rheumatology Research Unit, Repatriation General Hospital, and Flinders University; A-K. Ulfgren, PhD, Senior Scientist, Department of Rheumatology, Karolinska Hospital; D.J. Veale, MD, FRCP, Consultant Rheumatologist, Department of Rheumatology, St. Vincent's University Hospital, and The Conway Institute of Biomolecular and Biomedical Research, University College Dublin; P.P. Tak, MD, PhD, Professor of Medicine, Director of Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam.

Address reprint requests to Prof. B. Bresnihan, Department of Rheumatology, St. Vincent's University Hospital, Dublin 4, Ireland. E-mail: c.walsh@st-vincents.ie

Introduction

The characteristic microscopic appearances of rheumatoid arthritis (RA) include marked synovial lining layer hyperplasia containing fibroblast-like synoviocytes (FLS), and the accumulation of macrophages, T cells, B cells, plasma cells, natural killer (NK) cells, dendritic cells, mast cells, and neutrophils in the sublining layer. Many proinflammatory mediators and tissue-degrading products such as reactive oxygen and nitrogen species, prostaglandins, cytokines, autoantibodies, and proteases are secreted into the synovial compartment by both infiltrating and native cell populations[1]. The recognition of the synovium as the primary site of inflammation in RA has led to systematic clinical studies that included the evaluation of synovial tissue samples. Examination of peripheral blood and synovial fluid in RA has provided insights into the production of soluble mediators and mechanisms of inflammatory cell migration to different compartments. However, such studies provide only indirect information about events in synovial tissue, the critical therapeutic target in patients with RA.

Clinical studies of RA that included analysis of synovial tissue usually employed immunohistochemistry (IHC) to identify infiltrating cell populations and secreted proteins. Relevant methodological issues have been extensively evaluated. For example, when quantifying cellular infiltration, it was demonstrated that tissue samples obtained by closed-needle biopsy provided results that were similar to samples selected at the same time, and from the same joint, using arthroscopic guidance[2]. Moreover, many IHC markers of inflammation were equally expressed in large (knee) and small (wrist and metacarpophalangeal) joints[3]. The hetero-

geneous characteristics of synovial tissue have been highlighted, and the requirements for overcoming selection bias when quantifying inflammatory markers have been defined[4-6]. Different methods of quantification, including manual counting, semiquantitative, and digital, automated methodologies, have been compared[7,8].

Synovial Tissue Analysis in Clinical Trials

Early, open-label clinical studies demonstrated that the magnitude of the therapeutic response to standard disease modifying antirheumatic drugs (DMARD) in RA was associated with measurable changes in synovial tissue morphology after treatment[9-11]. Subsequent open-label studies in RA and other categories of chronic arthritis further highlighted specific effects of treatments such as methotrexate[12-14], corticosteroids[15,16], and infliximab[17-19] on mononuclear cell infiltration and on the expression of proinflammatory 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[1].

In recent years, synovial tissue was evaluated before and after treatment in several randomized clinical trials (RCT) of both DMARD and biologic agents[20-24]. 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 sublining macrophage infiltration and the magnitude of the clinical response[22-24]. In particular, one study was designed to identify the optimal IHC biomarker of clinical efficacy in a relatively small patient cohort following a short treatment duration[22]. Patients received either prednisolone according to the COBRA regimen[25] 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 polymerase chain reaction. 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 sublining layer macrophages as the optimal biomarker of the clinical response to corticosteroids[22]. Subsequently, the merit of using the number of sublining macrophages as a candidate biomarker was tested across a range of discrete interventions and kinetics[26]. Eighty-eight patients who participated in various RCT were evaluated in the same center, using standardized techniques. The treatments evaluated included methotrexate, leflunomide, prednisolone, infliximab, a specific chemokine inhibitor, and placebo. All patients had baseline and followup biopsies, and the Disease Activity Score 28 (DAS28) was performed. There was a significant correlation between the change in the number of macrophages and the change in DAS28. The change in sublining 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[24]. Thus, 48 hours after the first infusion of 3 mg/kg infliximab, a significant decrease in synovial tissue macrophage numbers was demonstrated. After one 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 sublining 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 sublining macrophage infiltration[27]. A subtherapeutic dose of anakinra (30 mg/day) also failed to alter synovial tissue morphology after 24 weeks[21]. 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 CD68+ macrophages[28]. Similarly, 2 independent studies have shown that interferon-ß therapy did not affect the number of sublining macrophages[29,30]. These observations suggest that therapies that fail to reduce the number of sublining macrophages are unlikely to be clinically effective.

In conclusion, the accumulated data from several studies suggest that sublining 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[31]. This was supported by a later cross-sectional study[32]. 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[33]. Lining layer macrophages are more highly activated than sublining macrophages, express greater amounts of interleukin 1 and tumor necrosis factor-α, and are thought to migrate into the expanding pannus that participates in the degradation of articular cartilage and subchondral bone[34]. Matrix metallo-

proteinase-1 (MMP-1) gene expression in both the lining layer and sublining layers was also strongly associated with the formation of new erosions[33]. In this study, the followup 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 sublining T cells and FLS, and deterioration in the Larsen radiographic score[35]. The followup period ranged between 38 and 72 months (mean 58 mo). Differences in the patient characteristics, the intervals between followup biopsies, and the different methods of determining joint damage may explain the discrepancy between the 2 studies. Taken together, and considering current concepts of disease pathogenesis, it is possible that accumulations of macrophages in critical numbers in the lining layer, and of cells expressing RANK-ligand (T cells and FLS), might predict joint damage, but that mediators of matrix degradation (e.g., MMP) 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 MMP in synovial tissue[12,20,22,24].

The Potential of Synovial Tissue Analysis in Future RCT

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 RCT, the systematic evaluation of changes in synovial tissue after commencing treatment enables identification of an early therapeutic effect, using relatively small numbers of patients. As potential advantages: 1. Direct proof of principle may be shown by molecular analysis of the specific effects of the intervention. 2. 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; and 3. 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:

- 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 (magnetic resonance) research groups are being developed in an attempt to enhance predictive and response indices in tissue; and
- Comparison between IHC and emerging technologies (e.g., quantitative polymerase chain reaction, microarray, tissue-based ELISA, proteomics) in measuring therapeutic effects is to be evaluated.

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