

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)

Key Indexing Terms:

SYNOVIAL TISSUE ANALYSIS

CLINICAL TRIALS

RHEUMATOID ARTHRITIS

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 populations¹. 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 guidance². Moreover, many IHC markers of inflammation were equally expressed in large (knee) and small (wrist and metacarpophalangeal) joints³. The hetero-

geneous characteristics of synovial tissue have been highlighted, and the requirements for overcoming selection bias when quantifying inflammatory markers have been defined⁴⁻⁶. 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 treatment⁹⁻¹¹. Subsequent open-label studies in RA and other categories of chronic arthritis further highlighted specific effects of treatments such as methotrexate¹²⁻¹⁴, corticosteroids^{15,16}, and infliximab¹⁷⁻¹⁹ 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 modalities¹.

In recent years, synovial tissue was evaluated before and after treatment in several randomized clinical trials (RCT) of both DMARD and biologic agents²⁰⁻²⁴. 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 response²²⁻²⁴. 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 duration²². Patients received either prednisolone according to the COBRA regimen²⁵ 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 corticosteroids²². Subsequently, the merit of using the number of sublining macrophages as a candidate biomarker was tested across a range of discrete interventions and kinetics²⁶. 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 improvement²⁴. 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 infiltration²⁷. A subtherapeutic dose of anakinra (30 mg/day) also failed to alter synovial tissue morphology after 24 weeks²¹. 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+ macrophages²⁸. Similarly, 2 independent studies have shown that interferon- β 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 damage³¹. This was supported by a later cross-sectional study³². 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 erosions³³. 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 bone³⁴. 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 erosions³³. 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 score³⁵. 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.

REFERENCES

1. Tak PP, Bresnihan B. The pathogenesis and prevention of joint damage in rheumatoid arthritis: Advances from synovial biopsy and tissue analysis. *Arthritis Rheum* 2000;43:2619-33.
2. Youssef PP, Kraan M, Breedveld F, et al. Quantitative microscopic analysis of inflammation in rheumatoid arthritis synovial membrane samples selected at arthroscopy compared with samples obtained blindly by needle biopsy. *Arthritis Rheum* 1998;41:663-9.
3. Kraan MC, Reece RJ, Smeets TJM, Veale DJ, Emery P, Tak PP. Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patients. Implications for pathogenesis and evaluation of treatment. *Arthritis Rheum* 2002;46:2034-8.
4. Rooney M, Condell D, Quinlan W, et al. Analysis of the histologic variation of synovitis in rheumatoid arthritis. *Arthritis Rheum* 1988;31:956-63.
5. Bresnihan B, Cunnane G, Youssef P, Yanni G, FitzGerald O, Mulherin D. Rheumatoid arthritis: proposals for the evaluation of tissue samples by quantitative analysis. *Br J Rheumatol* 1998;37:636-42.
6. Dolhain RJ, Ter Haar NT, De Kuiper R, et al. Distribution of T cells and signs of T-cell activation in the rheumatoid joint: implications for semiquantitative comparative histology. *Br J Rheumatol* 1998;37:324-30.
7. Cunnane G, Bjork L, Ulfgren A-K, et al. Quantitative analysis of synovial membrane inflammation: a comparison between automated and conventional microscopic measurement. *Ann Rheum Dis* 1999;58:493-9.
8. Kraan M, Haringman JJ, Ahern MJ, Breedveld FC, Smith MD, Tak PP. Quantification of the cell infiltrate in synovial tissue by digital image analysis. *Rheumatology Oxford* 2000;39:43-9.
9. Walters MT, Smith JL, Moore K, Evans PR, Cawley MI. An investigation of the action of disease modifying antirheumatic drugs on the rheumatoid synovial membrane: reduction in T lymphocyte subpopulations and HLA-DP and DQ antigen expression after gold or penicillamine therapy. *Ann Rheum Dis* 1987;46:7-16.
10. Rooney M, Whelan A, Feighery C, Bresnihan B. Changes in lymphocyte infiltration of the synovial membrane and the clinical course of rheumatoid arthritis. *Arthritis Rheum* 1989;32:361-9.
11. Yanni G, Farahat MNMR, Poston RN, Panayi GS. Intramuscular gold decreases cytokine expression and macrophage numbers in the rheumatoid synovial membrane. *Ann Rheum Dis* 1994;53:315-22.
12. Firestein GS, Paine MM, Boyle DL. Mechanism of methotrexate action in rheumatoid arthritis. Selective decrease in synovial collagenase gene expression. *Arthritis Rheum* 1994;37:193-200.
13. Dolhain RJEM, Tak PP, Dijkmans BAC, De Kuiper P, Breedveld FC, Miltenburg AMM. Methotrexate treatment reduced inflammatory cell numbers, expression of monokines and of adhesion molecules in synovial tissue of patients with rheumatoid arthritis. *Br J Rheumatol* 1998;37:502-8.
14. Kane D, Gogarty M, O'Leary J, et al. Methotrexate reduces synovial sub-lining layer inflammation and pro-inflammatory cytokine expression in psoriatic arthritis. *Arthritis Rheum*

- 2004;50:3286-95.
15. Youssef PP, Cormack J, Evill CA, et al. Neutrophil trafficking into inflamed joints in patients with rheumatoid arthritis and the effect of methylprednisolone. *Arthritis Rheum* 1996;39:216-25.
 16. Youssef PP, Haynes DR, Triantafillou S, et al. Effects of pulse methylprednisolone on inflammatory mediators in peripheral blood, synovial fluid, and synovial membrane in rheumatoid arthritis. *Arthritis Rheum* 1997;40:1400-8.
 17. Tak PP, Taylor PC, Breedveld FC, et al. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum* 1996;39:1077-81.
 18. Taylor PC, Peters AM, Paleolog E, et al. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor blockade in patients with rheumatoid arthritis. *Arthritis Rheum* 2000;43:38-47.
 19. Baeten D, Kruithof E, Van den Bosch F, et al. Immunomodulatory effects of anti-tumor necrosis factor alpha therapy on synovium in spondylarthropathy: histologic findings in eight patients from an open-label pilot study. *Arthritis Rheum* 2001;44:186-95.
 20. Kraan MC, Reece RJ, Barg EC, et al. Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. Findings in a prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. *Arthritis Rheum* 2000;43:1820-30.
 21. Cunnane G, Madigan A, Murphy E, FitzGerald O, Bresnihan B. The effects of treatment with interleukin-1 receptor antagonist on inflamed synovial membrane in rheumatoid arthritis. *Rheumatology Oxford* 2001;40:62-9.
 22. Gerlag DM, Haringman JJ, Smeets TJM, et al. Identification of biomarkers in synovial tissue associated with clinical improvement in rheumatoid arthritis. *Arthritis Rheum* 2004;50:3783-91.
 23. Haringman JJ, Kraan MC, Smeets TJM, Zwinderman KH, Tak PP. Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann Rheum Dis* 2003;62:715-21.
 24. Smeets TJM, Kraan MC, van Loon MC, Tak PP. Tumor necrosis factor alpha blockade reduces the synovial cell infiltrate early after initiation of treatment, but apparently not by induction of apoptosis in synovial tissue. *Arthritis Rheum* 2003;48:2155-62.
 25. Boers M, Verhoeven AC, Markusse HM, et al. Randomized comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. *Lancet* 1997;350:309-18.
 26. Haringman JJ, Gerlag DM, Zwinderman AH, et al. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;64:834-8.
 27. Smeets TJM, Kraan MC, Versendaal J, Breedveld FC, Tak PP. Analysis of serial synovial biopsies in patients with RA: Description of a control group without clinical improvement after treatment with interleukin 10 or placebo. *J Rheumatol* 1999;26:2089-93.
 28. Tak PP, Van der Lubbe PA, Cauli A, et al. Reduction of synovial inflammation after anti-CD4 monoclonal antibody treatment in early rheumatoid arthritis. *Arthritis Rheum* 1995;38:1457-65.
 29. Smeets TJM, Dayer JM, Kraan MC, et al. The effects of interferon-beta treatment on synovial inflammation and expression of metalloproteinases in patients with rheumatoid arthritis. *Arthritis Rheum* 2000;43:270-4.
 30. van Holten J, Pavelka K, Vencovsky J, et al. A multicenter, randomized, double-blind, placebo controlled phase II study of subcutaneously administered interferon beta 1a in the treatment of patients with active rheumatoid arthritis. *Ann Rheum Dis* 2005;64:64-9. Epub 2004 Jul 8.
 31. Yanni G, Whelan A, Feighery C, Bresnihan B. Synovial tissue macrophages and joint erosion in rheumatoid arthritis. *Ann Rheum Dis* 1994;53:39-44.
 32. Mulherin D, FitzGerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. *Arthritis Rheum* 1996;39:115-24.
 33. Cunnane G, FitzGerald O, Beeton C, Cawston TE, Bresnihan B. Early joint erosions and serum levels of matrix metalloproteinase 1, matrix metalloproteinase 3 and tissue inhibitor of metalloproteinases 1 in rheumatoid arthritis. *Arthritis Rheum* 2001;44:2263-74.
 34. Bresnihan B, Youssef P. Macrophages in rheumatoid arthritis. In: Burke B, Lewis CE, editors. *The macrophage*. Oxford: Oxford University Press;2002:391-433.
 35. Kraan MC, Haringman JJ, Weedon H, et al. T cells, fibroblast-like synoviocytes, and granzyme B+ cytotoxic cells are associated with joint damage in patients with recent onset rheumatoid arthritis. *Ann Rheum Dis* 2004;63:483-8.