

Synovial Tissue Analysis for the Discovery of Diagnostic and Prognostic Biomarkers in Patients with Early Arthritis

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ABSTRACT. Rheumatoid arthritis (RA) is a chronic disease of unspecified etiology that is manifest by persistent inflammation of the synovium. Considerable efforts have been undertaken globally to study the microenvironment of the inflamed synovium, with many encouraging and enlightening results that bring us closer to unmasking the precise etiologies of RA. Subsequent to these efforts, it has been discovered that CD68-positive macrophages present in abundance in the synovial sublining of the inflamed synovium rescind with treatments that induce clinical improvement in RA. Examination of serial synovial biopsies is now commonly used for screening purposes during early drug development, and the number of centers able to perform synovial tissue biopsy sampling according to standardized methods is increasing.

Having implemented the use of serial synovial tissue biopsies to evaluate the effects of new treatments on the group level in early proof of principle studies, it is the ambition of the OMERACT Synovial Tissue Group to identify synovial diagnostic and prognostic biomarkers that could be used in individual patients. Therefore, we started a prospective study termed the Synoviomics Project aimed at the identification of novel diagnostic and prognostic synovial biomarkers. We will use straightforward and powerful technologies to analyze patient material and assess clinical parameters to identify such biomarkers. These markers may be used in the future to identify patients who are at risk of having persistent and destructive disease and to start tailor-made targeted therapies in an early phase to prevent autonomous disease progression and irreversible joint damage. (J Rheumatol 2011;38:2068–72; doi:10.3899/jrheum.110426)

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This article is dedicated to the memory of Professor Barry Bresnihan, who played a pivotal role in the establishment of the OMERACT Special Interest Group on Synovial Analysis in Clinical Trials, and who died in 2010.

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Inflammation of the Synovium

In socioeconomic terms, rheumatoid arthritis (RA) is the most common and most important of the inflammatory arthritides. It is a debilitating chronic erosive disease that affects 1–2% of the population worldwide. Smoking tobacco, being female, and expressing certain genetic alleles are among the established risk factors for developing RA. At present, delay in diagnosis may result from lack of definitive biomarkers or failure to meet current diagnostic criteria. Therefore, patient-specific treatment may not be introduced, and hence accurate novel biomarkers are needed to enable early diagnosis. Synovial inflammation is the hallmark of the disease. The inflamed synovium expands into and destroys the underlying cartilage and bone, resulting in irreversible erosion of the bone and eventually in loss of normal joint architecture and disability¹.

Patients with RA should be classified in an early stage of the disease to allow initiation of appropriate treatment, since early treatment has been proven to reduce or halt joint destruction². Of importance, a subset of the patients with early arthritis cannot be classified during early disease due to the heterogeneity of the disease and the lack of definitive diagnostic markers, and are diagnosed as patients with undifferentiated

arthritis. Therefore, patient-specific treatment may not be initiated. In addition, arthritides other than RA may have a persistent and destructive course, and aggressive treatment may be required in these patients as well. Thus, there is a need for the identification of biomarkers predictive of diagnosis, as well as persistent and destructive disease, to start tailor-made targeted therapies in an early phase to prevent autonomous disease progression and irreversible joint damage.

Evidence suggests that the phenotype described as RA may be the result of different pathogenetic pathways³. As synovitis is the primary pathogenic event underlying signs and symptoms of arthritis in RA, we endeavor to better understand its pathology. Autoimmune activation, coupled with upregulation of proinflammatory cytokines and mobilization of inflammatory cells to the synovium, plays a considerable role, but the precise etiology of the disease is as yet unclear.

In the healthy state, the synovial tissue is composed of 1 to 3 layers of specialized columnar cells called fibroblast-like synoviocytes with interspersed macrophages⁴. It is divided into an intimal lining layer without an underlying basement membrane, and a synovial sublining layer that is continuous with the joint capsule. Its physiologic function is to secrete synovial fluid, which lubricates the joint and nourishes the avascular cartilage. Microscopic analysis of the synovium has given us some insight into the pathogenesis of RA. Rheumatoid synovial tissue is hypertrophic and edematous and is characterized by marked intimal lining hyperplasia and by accumulation of T lymphocytes, plasma cells, macrophages, B lymphocytes, neutrophils, mast cells, natural killer cells, and dendritic cells in the synovial sublining⁵. Villous projections of synovial tissue protrude into the synovial cavity and erode into the underlying cartilage and bone. Neovascularization, the development of new blood vessels, within the inflamed synovium facilitates the migration of leukocytes and contributes to the perpetuation of this chronic disease⁶. Pannus is the name used to describe hypertrophic synovial tissue near the synovium-cartilage junction. Inflamed joints are known to be hypoxic^{7,8}, and it is thought that the hypoxic milieu of the inflamed pannus is one of the stimuli for neovascularization via activation of molecular hypoxic pathways such as HIF 1 α . Targeting neovascularization might provide a novel therapeutic strategy in RA⁹. These findings illustrate that descriptive studies of the rheumatoid synovium help us to understand the events that take place *in vivo* and complement experimental animal studies as well as *in vitro* studies. Synovial tissue analysis may thus lead to the discovery of diagnostic and prognostic biomarkers in patients with early arthritis, which is paramount to patient-specific care, given that early intervention may prevent subsequent disability.

What Specifically Have We Learned from Analysis of the Synovium in RA?

Several centers worldwide now sample the synovium of people affected by early inflammatory arthritis. We have recently

developed a consensus on the techniques used to obtain and process synovial biopsy samples¹⁰. There are 3 main techniques for obtaining synovial biopsy samples: arthroscopic, ultrasound-guided, and blind needle synovial biopsy sampling¹¹. They have all been validated as methods of retrieval of synovial tissue for RA research, yet the former 2 are favored for proof-of-concept experiments.

Arthroscopic synovial biopsy sampling confers the advantage of direct visualization of the inflamed synovium, whereas ultrasound depicts synovial swelling and bone erosions on a gray-scale, and with the use of power Doppler technology reveals active synovitis, allowing the operating technician to selectively sample the inflamed synovium¹². Both may be undertaken under local anesthetic in a sterile environment and both have an acceptable adverse event profile. Inspection of inflamed synovial tissue has enhanced our understanding of the function of various cell types and mediators in RA and has provided some insight into its pathogenesis.

The synovium has been studied at macroscopic, microscopic, and molecular levels. The blood vessels of the inflamed rheumatoid synovium tend to be straight branching in RA, as opposed to the tortuous pattern seen in spondyloarthritides¹³. It is known that the quantity of proinflammatory cytokines and inflammatory cells is reduced in the synovial membrane of treated RA patients with low disease activity¹⁴. Pretreatment synovial inflammation and tumor necrosis factor- α expression correlated with therapeutic response to infliximab^{15,16}, and it has been shown that synovial cell infiltration, particularly by macrophages, and macrophage-derived cytokine expression were reduced after prednisolone therapy, with a significant correlation to beneficial clinical effect¹⁷. B cell depletion therapy has been shown to deplete synovial B lymphocyte populations in patients with refractory RA who had an excellent response to therapy^{18,19}.

CD68-positive macrophages are significantly upregulated in the synovial sublining layer of inflamed rheumatoid synovial tissue compared to healthy synovium, and several experiments have consistently shown that the quantity of CD68 macrophages in the synovial sublining (CD68sl) is reduced concurrent with a reduction in disease activity, as measured by the Disease Activity Score (DAS)²⁰. It has also been shown that when therapy has failed and inflammation persists, CD68sl do not decrease in number, further supporting its use as an accurate biomarker that can be used on the group level to distinguish effective from ineffective treatment²¹ (see Table 1 for an overview of studies showing the relationship between changes in DAS28 and CD68sl after treatment).

CD68sl expression was proposed as a marker of response to therapy in RA by the OMERACT Special Interest Group on Synovial Analysis in Clinical Trials at OMERACT 7. Trials undertaken at the Academic Medical Center (AMC) Amsterdam and St. Vincent's University Hospital Dublin confirmed a consistent correlation between the mean change in CD68sl and the mean change in Disease Activity Score 28

Table 1. Overview of therapies that showed a correlation between infiltration of CD68-positive macrophages in RA synovial tissue and disease activity and response to treatment.

Treatment
Leflunomide ²³
Methotrexate ²³
Infliximab ²⁴
CCR1-antagonist ²⁵
Prednisolone ¹⁷
Anti-CCL2 antibody ²⁶
C5aR-antagonist ²⁷
Rituximab ²⁸
Anti-CCR2 antibody ²⁹
CCR5 antagonist ³⁰

CCR: CC chemokine receptor; CCL: CC chemokine ligand.

(DAS28) across different centers²². At OMERACT 9, it was agreed that arthroscopic synovial biopsy sampling in clinical trials is both viable and safe. Further, it was decided that CD68sl expression in synovial tissue provides an accurate reflection of disease activity that is superior to clinical evaluation as it is less susceptible to both placebo effect and expectation bias. CD68sl may thus be reliably used as a tool for assessment of therapeutic efficacy of novel treatments²².

We therefore recently started a new dynamic fellowship aimed at implementation of the concept of early, high density of data, proof-of-principle studies in RA around the world. This fellowship is focused on good clinical practice, clinical trial design and conduct, synovial biopsy, musculoskeletal ultrasound, and core laboratory techniques.

At OMERACT 10, the Special Interest Group on Synovial Analysis in Clinical Trials presented a new program aimed at identification of synovial diagnostic and prognostic biomarkers that could be used in individual patients, called the Synoviomics Project.

The Synoviomics Project

Objectives. Since 2002, a cohort of patients with early arthritis has been gathered at AMC in Amsterdam; this venture, aimed at the identification of novel diagnostic and prognostic biomarkers, has been termed the "Synoviomics Project." The immediate goal of the project is to provide insight into the pathogenesis of various forms of arthritis, especially RA. Patients with inflammatory arthritis will be scrutinized at clinical, macroscopic, molecular, and genetic levels. Ultimately, the working group hopes to identify new diagnostic, prognostic, and therapeutic targets to enable tailor-made treatment that may lead to the prevention of joint damage and disability in the long term. These aims are to be achieved specifically as follows.

1. Generation of sample libraries of gene expression analysis in conjunction with a database containing all biological and clinical data of recruited patients;
2. Prospective review of a cohort of patients with newly diagnosed arthritis; and

3. Selection of genes and proteins of interest to be investigated further.

In addition to diagnostic shortcomings, criteria permitting prediction of disease evolution in very early arthritis are imprecise. Identification of patients with early arthritis who will develop persistent and/or destructive disease is paramount for developing effective tailor-made therapeutic strategies^{31,32,33}. Thus, an important goal of close monitoring of patients in the early phase of their disease is to identify reliable markers predictive of joint damage. First, finding biomarkers in peripheral blood is of great interest and is a more feasible and less invasive technique than taking synovial biopsies. However, we do not want to restrict biomarker research to the serum, but favor a combination of soluble as well as synovial tissue biomarker findings, since the synovial tissue is the main target of inflammation in RA. These 2 compartments of the immune system are in close contact with each other and, according to the current number of synovial tissue studies performed, synovial tissue biopsy procedures and analyses are becoming more and more available worldwide.

Thanks to the new high-throughput technologies and analyses, researchers are quickly building up detailed portraits of the patterns of gene activity associated with various types of inflammatory disease. This knowledge promises to transform clinical decision-making, boost treatment success rates, and lead to new targeted drugs for use with truly customized therapeutic programs. Expression profiling has already shown its usefulness in identifying genes in specific cell types under defined conditions and in establishing characteristic patterns of gene expression in a variety of diseases. Several studies have shown that DNA array technology used to study gene expression in RA is a feasible approach, and gene expression analysis has revealed the existence of different pathological subtypes of affected synovium in RA^{34,35,36}. Since it is becoming more apparent that there are many factors involved in the onset and perpetuation of RA, and that the interactions between those factors are extremely complex, an essential effort has to be made to avoid a vision that is too restrictive. To increase the understanding of the mechanisms involved in such conditions and, consequently, to identify new therapeutic targets and to develop novel diagnostic tools, it is essential to do an exploratory, precise analysis of the genes expressed in the tissue at the mRNA and protein level. The microarrays currently used contain probes for several thousands of different genes, having the advantage that it is not necessary to hypothesize in advance what the important genes or mechanisms would be. In fact, it allows obtaining a broader and less biased view of the cellular response. It is therefore important to analyze the gene expression profile in synovial tissue of patients with early arthritis with respect to diagnostic and prognostic outcome. After gene expression profiling, genes of interest may be validated in an independent cohort. This might give us insight into genes involved in the pathogenesis and persistence of RA to establish tailor-made treatment for the

individual patient and therefore improve efficiency of health-care. The critical factor in this program is clear definition of patient subgroups.

Examination of a cohort of early arthritis patients and creation of a database with the cumulative clinical data as well as data from histology, DNA arrays, mRNA arrays, and proteomics is an instrumental resource for investigating differences in synovial tissue comparing several inflammatory joint diseases and comparing patients with persistent self-limiting disease and persistent disease, either non-erosive or erosive.

MATERIALS AND METHODS

Disease-modifying antirheumatic drug (DMARD)-naive patients with early arthritis with at least one swollen joint suitable for synovial biopsy and disease duration of less than one year are included in this study. The study was approved by the Medical Ethics Committee of the Academic Medical Center/University of Amsterdam and participating centers in Ireland, Sweden, and the United Kingdom, and is performed according to the Declaration of Helsinki. All patients give written informed consent. At baseline and annual visits over 5 years, demographic and clinical data are collected, blood and urine samples are gathered, and radiographs of the joints are obtained. In addition, patients undergo dynamic contrast-enhanced MR imaging and synovial biopsy sampling at baseline¹¹. In patients who fulfil the classification criteria for RA [previously the 1987 American College of Rheumatology (ACR) criteria; currently the 2010 ACR/European League Against Rheumatism criteria]^{37,38} after 6 months of followup, synovial biopsy sampling is repeated to determine the role of factors involved in different phases of synovial inflammation and to evaluate the effect of antirheumatic treatment on the synovial tissue infiltrate and gene expression in the RA synovium. After inclusion, patients receive standard care according to their treating rheumatologist and are allowed to participate in clinical trials. Data on treatment regimens are carefully documented.

Patients will be followed over time with, as key endpoints, classification according to diagnosis (according to the disease-specific diagnostic criteria) and outcome (self-limiting disease, persistent disease, or persistent destructive disease)³⁹ after 2 and 5 years. In an exploratory way, we will also examine erosive disease and joint space narrowing separately. The molecular features of synovial tissue samples obtained at baseline will be correlated with the clinical data after 2 and 5 years of followup to identify diagnostic and prognostic biomarkers.

RESULTS

Since the Synoviomics Project started, more than 270 patients with early arthritis have been included at the AMC. To increase the number of patients, a collaborative network has been set up with other centers in Europe (St. Vincent's University Hospital, Dublin; Barts and the London School of Medicine, London; University of Birmingham; Karolinska University Hospital; University of Newcastle upon Tyne). The first analyses of the Synoviomics Project have been performed, and these results will be published in 2011.

Moreover, at OMERACT 10 the Special Interest Group on Synovial Analysis in Clinical Trials discussed the literature review and first results and decided on the following aims for the next period:

1. Develop a consensus on techniques used to biopsy, process, preserve, and quantify synovial tissue for the purpose of clinical research.
2. Discover the inherent differences between the synovial tis-

sue from patients with inflammatory arthritis with erosive disease versus disease that results only in joint space narrowing.

3. Correlate the results of 1 and 2 with patient clinical disease activity scores, radiographic data, and patient-reported functional outcomes.

The first aim focuses on "feasibility," and the latter 2 will be of importance for determining their "truth" and "discrimination," key components of the OMERACT filter⁴⁰. Together, by the proposed analyses in early arthritis patient samples, we aim to obtain not only pivotal information about the pathogenesis of various forms of arthritis, but also for developing novel diagnostic tools and identifying prognostic biomarkers that may help develop new treatment regimens and enable patient-specific care.

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