EULAR Synovitis Study Group: Summary of activities 2010-2011

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What specifically have we learnt from the analysis of the synovium in RA?
Several centres worldwide now sample the synovium of people affected by early inflammatory arthritis. There are three main techniques used for obtaining synovial biopsy samples: arthroscopic, ultrasound guided as well as blind needle synovial biopsy sampling (1). They have all been validated as methods of retrieval of synovial tissue for the purpose of research of RA, yet the former two are favoured 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 grayscale and with the use of power Doppler technology reveals active synovitis, allowing the operating technician to selectively sample the inflamed synovium. Both may be undertaken under local anaesthetic 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 the spondyloarthritides (2). It is known that the quantity of pro-inflammatory cytokines and inflammatory cells is reduced in the synovial membrane of a treated rheumatoid arthritis patient with low disease activity (3). Pre-treatment synovial inflammation, TNF alpha expression and the presence of lymphocyte aggregates correlated with therapeutic response to infliximab (4-6), 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 effect (7). B cell depletion therapy has been shown to deplete synovial B lymphocyte populations in refractory RA patients who had an excellent response to therapy (8-12). 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) (13;14). 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 treatment from ineffective treatment (14). Trials undertaken at the Academic Medical Center in Amsterdam (AMC) 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 (DAS28) across different centres (15). At OMERACT 9 it was agreed that arthroscopic synovial biopsy in clinical trials is both viable and safe. Furthermore 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 the placebo effect and investigator bias. CD68sl may thus be reliably used as a tool for assessment of therapeutic efficacy of novel (15). In collaboration with Prof. Iain McInnes and sponsored by EU-Autocure and NovoNordisk, we have set up a 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.

The Synovial Tissue Group has also started a program aimed at identification of synovial diagnostic and prognostic biomarkers that could be used in individual patients, called the ‘Synoviomics project’.

The Synoviomics Project
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The immediate goal of the ‘Synoviomics project’ is to provide insight into the pathogenesis of various forms of arthritis, especially RA. Patients suffering from 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. These aims are to be achieved specifically by

1. The generation of sample libraries of gene expression analysis in conjunction with a database containing all biological and clinical data of patients recruited.
2. The prospective review of a cohort of newly diagnosed arthritis patients.
3. The selection of genes and proteins of interest to be investigated further.

Additional to the diagnostic shortcomings, criteria permitting the prediction of disease evolution in very early arthritis are imprecise. Identification of early arthritis patients who will develop persistent and/or destructive disease is paramount to develop tailor-made targeted therapies, which could be used to prevent disease progression and irreversible joint damage. 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 a more feasible and less invasive technique than taking synovial biopsies. However, we do not want to restrict biomarker research to the serum but favour a combination of soluble as well as synovial tissue biomarker finding since the synovial tissue is the main target of inflammation in RA. These two 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 analysis, 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. 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 expressed genes in the tissue at the mRNA and protein level. The current microarrays 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 early arthritis patients 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 healthcare. 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 in biopsy samples from patients with persistent, erosive disease compared with self-limiting and non-erosive disease, respectively. Since 2002, a cohort involving these parameters 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’. Disease-modifying antirheumatic drug (DMARD) naïve early arthritis patients with at least one swollen joint suitable for synovial biopsy and a disease duration of less than one year are included in this study. At baseline and annual visits over 2 years, demographic and clinical data are collected, diagnosis is established, blood and urine samples gathered and radiographs of the joints are obtained. In addition, all patients undergo dynamic
contrast material-enhanced MR imaging (DCE-MRI). Patients undergo synovial biopsy sampling at baseline [9]. In patients who already fulfill the ACR classification criteria for RA, synovial biopsy sampling is repeated after 6 months to determine the role of factors involved in different phases of synovial inflammation and to evaluate the effect of anti-rheumatic treatment on the synovial tissue infiltrate and gene expression in the RA synovium. After two years of follow up patients are classified according to outcome: self-limiting disease, persistent disease, or persistent erosive disease. Patients are also classified according to the final diagnosis after follow up. The molecular features of synovial tissue samples obtained at baseline will be correlated with the clinical data after two years of follow up to identify diagnostic and prognostic biomarkers.

Since the Synoviomics project has started, more than 270 early arthritis patients have been included at AMC. To increase the number of patients, a collaborative network has been set up with other centres in Europe (St Vincent's University Hospital, Dublin (70 patients included); University of Birmingham (30 patients included); Karolinska University Hospital (first patients included). The Barts and the London School of Medicine (London) has started a comparable program. Analysis of the first 93 patients with early arthritis at AMC focused on the role of lymphocyte aggregates in early arthritis (16). After 2 years of follow-up, definitive diagnosis and clinical outcome were assessed. Size of synovial lymphocyte aggregates was graded (score 1-3). Lymphoid neogenesis was defined by the presence of grade ≥2 aggregates and subclassified based on the presence of follicular dendritic cells. This study showed that the presence of lymphocyte aggregates is a dynamic phenomenon related to the degree of synovitis and can be detected in different forms of early arthritis. This feature does not appear to be related to clinical outcome. Current studies focus on the role of markers of neoangiogenesis and intracellular signalling pathways in early arthritis.

In conclusion, analysis of early arthritis patient samples from different compartments including the synovial tissue and peripheral blood by high throughput techniques could not only provide pivotal information about the pathogenesis of various forms of arthritis, but may also help to develop novel diagnostic tools and to identify prognostic markers.
Reference List


