The Journal of Rheumatology Volume 38, no. 9

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

MARIA J.H. de HAIR, LEONARD C. HARTY, DANIELLE M. GERLAG, COSTANTINO PITZALIS, DOUGLAS J. VEALE and PAUL P. TAK

J Rheumatol 2011;38;2068-2072
http://www.jrheum.org/content/38/9/2068

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

MARIA J.H. de HAIR, LEONARD C. HARTY, DANIELLE M. GERLAG, COSTANTINO PITZALIS, DOUGLAS J. VEALE, and PAUL P. TAK

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)

Key Indexing Terms:
EARLY ARTHRITIS SYNOVIUM BIOMARKER

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.

From the Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands; Centre for Arthritis Research and Education, Dublin Academic Health Care, St. Vincent's University Hospital, Dublin, Ireland; and the William Harvey Research Institute, Barts and the London School of Medicine, London, UK.

Supported by the OMERACT Special Interest Group on Synovial Analysis in Clinical Trials, the EULAR Synovitis Study Group and the European Community's FP6 funding (Autocure).

M.J.H. de Hair, MD, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam; L.C. Harty, MD, Centre for Arthritis Research and Education, Dublin Academic Health Care, St. Vincent's University Hospital; D.M. Gerlag, MD, PhD, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam; C. Pitzalis, MD, PhD, FRCP, William Harvey Research Institute, Barts and the London School of Medicine; D.J. Veale, MD, FRCP, Centre for Arthritis Research and Education, Dublin Academic Health Care, St. Vincent's University Hospital; P.P. Tak, MD, PhD, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam.

Dr. de Hair and Dr. Harty contributed equally and should be considered co-first authors of this article.

Address correspondence to Prof. Tak; E-mail: P.P.Tak@amc.uva.nl,

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 disability[1].

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 destruction[2]. 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 pathways[3]. 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 macrophages[4]. 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 sublining[5]. Villous projections of synovial tissue protrude into the synovial cavity and erode into the underlying cartilage and bone. Neoangiogenesis, the development of new blood vessels, within the inflamed synovium facilitates the migration of leukocytes and contributes to the perpetuation of this chronic disease[6]. 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 neoangiogenesis via activation of molecular hypoxic pathways such as HIF 1α. Targeting neoangiogenesis might provide a novel therapeutic strategy in RA[9]. 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 samples[10]. There are 3 main techniques for obtaining synovial biopsy samples: arthroscopic, ultrasound-guided, and blind needle synovial biopsy sampling[11]. 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 synovium[12]. 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 spondyloarthritides[13]. 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 activity[14]. 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 effect[17]. 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 treatment[21] (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 centers[22]. 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 treatments[22].

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 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 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 baseline[11]. 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 tissue 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 filter[40]. 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.

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. Finckh A, Liang MH, van Herckenrode CM, de Pablo P. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. Arthritis Rheum 2006;55:864-72.
3. Tak PP. Analyzing synovial tissue samples. What can we learn about early rheumatoid arthritis, the heterogeneity of the disease, and the effects of treatment? J Rheumatol Suppl 2005;72:25-6.
4. Smith MD, Barg E, Weedon H, Papengelis V, Smeets T, Tak PP, et al. Microarchitecture and protective mechanisms in synovial tissue from clinically and arthroscopically normal knee joints. Ann Rheum Dis 2003;62:303-7.
5. Tak PP, Smeets TJ, Daha MR, Kluin PM, Meijers KA, Brand R, et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 1997;40:217-25.
6. Fearon U, Veale DJ. Angiogenesis in arthritis: methodological and analytical details. Methods Mol Med 2007;135:343-57.
7. Kennedy A, Ng CT, Biniecka M, Saber T, Taylor C, O'Sullivan J, et al. Angiogenesis and blood vessel stability in inflammatory arthritis. Arthritis Rheum 2010;62:711-21.
8. Biniecka M, Kennedy A, Fearon U, Ng CT, Veale DJ, O'Sullivan JN. Oxidative damage in synovial tissue is associated with in vivo hypoxic status in the arthritic joint. Ann Rheum Dis 2010;69:1172-8.
9. Gerlag DM, Borges E, Tak PP, Ellerby HM, Bredesen DE, Pasqualini R, et al. Suppression of murine collagen-induced arthritis by targeted apoptosis of synovial neovasculature. Arthritis Res 2001;3:357-61.
10. van de Sande MG, Gerlag DM, Lodde BM, van Baarsen LG, Alivernini S, Codullo V, et al. Evaluating antirheumatic treatment using synovial biopsy: a recommendation for standardisation to be used in clinical trials. Ann Rheum Dis 2011;70:423-7.
11. Gerlag DM, Tak PP. How to perform and analyse synovial biopsies. Best Pract Res Clin Rheumatol 2009;23:221-32.
12. Scire CA, Epis O, Codullo V, Humby F, Morbini P, Manzo A, et al. Immunohistological assessment of the synovial tissue in small joints in rheumatoid arthritis: validation of a minimally invasive ultrasound-guided synovial biopsy procedure. Arthritis Res Ther 2007;9:R101.
13. Reece RJ, Canete JD, Parsons WJ, Emery P, Veale DJ. Distinct vascular patterns of early synovitis in psoriatic, reactive, and rheumatoid arthritis. Arthritis Rheum 1999;42:1481-4.
14. Gerlag DM, Tak PP. Novel approaches for the treatment of

rheumatoid arthritis: lessons from the evaluation of synovial biomarkers in clinical trials. Best Pract Res Clin Rheumatol 2008;22:311-23.
15. van der Pouw Kraan TC, Wijbrandts CA, van Baarsen LG, Rustenburg F, Baggen JM, Verweij CL, et al. Responsiveness to anti-tumour necrosis factor alpha therapy is related to pre-treatment tissue inflammation levels in rheumatoid arthritis patients. Ann Rheum Dis 2008;67:563-6.
16. Wijbrandts CA, Dijkgraaf MG, Kraan MC, Vinkenoog M, Smeets TJ, Dinant H, et al. The clinical response to infliximab in rheumatoid arthritis is in part dependent on pretreatment tumour necrosis factor alpha expression in the synovium. Ann Rheum Dis 2008;67:1139-44.
17. Gerlag DM, Haringman JJ, Smeets TJ, Zwinderman AH, Kraan MC, Laud PJ, et al. Effects of oral prednisolone on biomarkers in synovial tissue and clinical improvement in rheumatoid arthritis. Arthritis Rheum 2004;50:3783-91.
18. Kavanaugh A, Rosengren S, Lee SJ, Hammaker D, Firestein GS, Kalunian K, et al. Assessment of rituximab's immunomodulatory synovial effects (ARISE trial). 1: clinical and synovial biomarker results. Ann Rheum Dis 2008;67:402-8.
19. Vos K, Thurlings RM, Wijbrandts CA, van Schaardenburg D, Gerlag DM, Tak PP. Early effects of rituximab on the synovial cell infiltrate in patients with rheumatoid arthritis. Arthritis Rheum 2007;56:772-8.
20. Haringman JJ, Gerlag DM, Zwinderman AH, Smeets TJ, Kraan MC, Baeten D, et al. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. Ann Rheum Dis 2005;64:834-8.
21. Wijbrandts CA, Vergunst CE, Haringman JJ, Gerlag DM, Smeets TJ, Tak PP. Absence of changes in the number of synovial sublining macrophages after ineffective treatment for rheumatoid arthritis: Implications for use of synovial sublining macrophages as a biomarker. Arthritis Rheum 2007;56:3869-71.
22. Bresnihan B, Pontifex E, Thurlings RM, Vinkenoog M, El-Gabalawy H, Fearon U, et al. Synovial tissue sublining CD68 expression is a biomarker of therapeutic response in rheumatoid arthritis clinical trials: consistency across centers. J Rheumatol 2009;36:1800-2.
23. Kraan MC, Reece RJ, Barg EC, Smeets TJ, Farnell J, Rosenburg R, 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.
24. Smeets TJ, Kraan MC, van Loon ME, 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. Haringman JJ, Kraan MC, Smeets TJ, 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.
26. Haringman JJ, Gerlag DM, Smeets TJ, Baeten D, van den Bosch F, Bresnihan B, et al. A randomized controlled trial with an anti-CCL2 (anti-monocyte chemotactic protein 1) monoclonal antibody in patients with rheumatoid arthritis. Arthritis Rheum 2006;54:2387-92.
27. Vergunst CE, Gerlag DM, Dinant H, Schulz L, Vinkenoog M, Smeets TJ, et al. Blocking the receptor for C5a in patients with rheumatoid arthritis does not reduce synovial inflammation. Rheumatology 2007;46:1773-8.
28. Thurlings RM, Vos K, Wijbrandts CA, Zwinderman AH, Gerlag DM, Tak PP. Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. Ann Rheum Dis 2008;67:917-25.
29. Vergunst CE, Gerlag DM, Lopatinskaya L, Klareskog L, Smith MD, van den Bosch F, et al. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. Arthritis Rheum 2008;58:1931-9.
30. van Kuijk AW, Vergunst CE, Gerlag DM, Bresnihan B, Gomez-Reino JJ, Rouzier R, et al. CCR5 blockade in rheumatoid arthritis: a randomised, double-blind, placebo-controlled clinical trial. Ann Rheum Dis 2010;69:2013-6.
31. Drossaers-Bakker KW, Zwinderman AH, Vliet Vlieland TP, Van Zeben D, Vos K, Breedveld FC, et al. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. Arthritis Rheum 2002;47:383-90.
32. Mottonen T, Hannonen P, Korpela M, Nissila M, Kautiainen H, Ilonen J, et al. Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatic drug therapy in early rheumatoid arthritis. Arthritis Rheum 2002;46:894-8.
33. Scott DL. The diagnosis and prognosis of early arthritis: rationale for new prognostic criteria. Arthritis Rheum 2002;46:286-90.
34. Haupl T, Stuhlmuller B, Grutzkau A, Radbruch A, Burmester GR. Does gene expression analysis inform us in rheumatoid arthritis? Ann Rheum Dis 2010;69 Suppl 1:i37-i42.
35. van Baarsen LG, Wijbrandts CA, Timmer TC, van der Pouw Kraan TC, Tak PP, Verweij CL. Synovial tissue heterogeneity in rheumatoid arthritis in relationship to disease activity and biomarkers in peripheral blood. Arthritis Rheum 2010;62:1602-7.
36. van der Pouw Kraan TC, van Gaalen FA, Kasperkovitz PV, Verbeet NL, Smeets TJ, Kraan MC, et al. Rheumatoid arthritis is a heterogeneous disease: evidence for differences in the activation of the STAT-1 pathway between rheumatoid tissues. Arthritis Rheum 2003;48:2132-45.
37. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-81.
38. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO III, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010;69:1580-8.
39. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002;46:357-65.
40. Boers M, Brooks P, Strand CV, Tugwell P. The OMERACT filter for Outcome Measures in Rheumatology. J Rheumatol 1998;25:198-9.