Assessment of synovitis to predict bone erosions in rheumatoid arthritis

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Abstract: Although rheumatoid arthritis (RA) is traditionally considered as the prototype of destructive arthritis, the course of the disease varies considerably, with some patients experiencing more rapid progression of joint damage and disability than others. Given the increasing availability of treatment targets and options, timely recognition of individual's outcomes could allow therapeutic allocation according to personalized benefit–risk profiles. Research efforts are thus increasingly focused at discovering predictive markers that could identify patients with aggressive, rapidly progressive disease and poor prognosis. As joint destruction in RA is the result of the cumulative burden of inflammation, variables reflecting the severity of synovitis and its persistence over time might refine our ability to build early prognostic algorithms. The goal of this article is to review the clinical implications of the assessment of synovitis in relation to radiographic outcomes. Traditional and novel assessment tools will be discussed, including clinical measures, imaging techniques and tissue biomarkers. Achievements in the field of synovial tissue analysis and peripheral blood biomarkers of synovitis represent only the first steps of ongoing progress, which still need to be integrated into the phenotypic heterogeneity of RA.

Keywords: rheumatoid arthritis, synovitis, bone erosion, biomarker

Introduction

Rheumatoid arthritis (RA) is traditionally considered as the prototype of destructive arthritis. Chronic inflammation of the synovial membrane in RA indeed results in bone and cartilage resorption through the production of molecules that support the differentiation and activation of osteoclasts as well as enzymes which degrade articular cartilage and bone [Schett, 2007; Walsh and Gravallese, 2010]. The central importance of joint remodeling processes in RA pathology is highlighted by the fact that the assessment of structural damage using imaging techniques is a major diagnostic, monitoring, and outcome parameter in both clinical trials and routine clinical practice [Lillegraven *et al.* 2012]. Joint destruction often begins very early and progresses rapidly within the first years from symptoms onset [Lindqvist *et al.* 2003; Machold *et al.* 2007]. The severity of bone and cartilage damage does have functional implications at least in the later stages of the disease, as suggested by multiple studies demonstrating the association between increasing joint destruction over time and progressive decline in physical function [Drossaers-Bakker *et al.* 1999; Scott *et al.* 2000; Welsing *et al.* 2001]. Preventing joint damage is therefore a key measure of current and novel therapies and treatment strategies in RA.

Despite common clinical and pathophysiological features, RA appears to consist of a variety of different phenotypes in terms of clinical expression and long-term course, including the extent and rate of progression of joint damage. Studies on very early arthritis (≤3 months from symptom onset) have shown that up to 20% of RA patients already present with erosions at baseline despite early referral [Nell *et al.* 2004; Machold *et al.* 2007], and substantial structural damage further develops even during disease-modifying antirheumatic drug (DMARD) therapy [Machold *et al.* 2007; Rezaei *et al.* 2012]. Such a proportion may be even higher in selected cohorts of patients with aggressive RA eligible for randomized clinical trials, such as in the BeSt study, where erosive disease is observed in up to 70% of the cases [Visser

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et al. 2010]. On the other hand, rheumatologists in daily clinical practice well appreciate that a considerable number of patients does not experience any erosion even after long-term disease. Following the greater availability of treatment targets and options, the prospect of distinguishing which patient with early RA is to run a severe disease course and which is not is, at present, one of the main challenges in the management of RA. Early predictors of disease outcome would indeed ideally channel aggressive treatments to patients most at risk, thus optimizing efficacy, adverse events and socioeconomic costs.

A number of demographic, immunologic and genetic factors have been variably associated with more severe outcomes in RA at the population level, including age, gender, autoantibody status and human leukocyte antigen (HLA) genotyping [Markatseli *et al.* 2010]. In particular, positivity for anticitrullinated protein antibodies (ACPAs) is currently recognized as one of the strongest independent predictors of erosive disease [Meyer *et al.* 2003; Forslind *et al.* 2004; van der Helm-van Mil *et al.* 2005; Syversen *et al.* 2008]. In the study by Syversen and colleagues [Syversen *et al.* 2008], a positive ACPA test predicted radiographic progression after 10 years of follow up with an odds ratio (OR) of 4 in a multiple regression model also including female gender, high erythrocyte sedimentation rate (ESR) and IgM rheumatoid factor (RF) positivity. Combined together, the probability of radiographic progression was 92.5% in females with a positive ACPA and IgM RF test and a high ESR [Syversen *et al.* 2008]. Despite excellent sensitivity, however, specificity may be unsatisfactory. A recent study by Liao and colleagues [Liao *et al.* 2011] failed to demonstrate a significant value of the ACPA test in predicting erosion-free status after 2 years of follow up. Many efforts are thus being made in the search for additional markers able to identify different radiographic outcomes since the earliest stages of the disease. As joint destruction in RA is the result of the cumulative burden of inflammation, predictive factors of radiographic progression may be searched within variables associated with the severity of inflammation and its persistence over time. Here we focus on new and renewed data which make the assessment of synovitis through clinical, imaging, histopathologic and serologic examinations, a valuable tool to look into disease heterogeneity in RA.

Clinical assessment

Clinical evaluation of involved joints through formal joint counts remains the cornerstone of the quantitative assessment of inflammation in RA. Physical examination *per se* is thus expected to provide important information on different radiological outcomes of the disease. Accordingly, cumulative swollen joint counts (SJCs) have repeatedly been shown to be associated with joint damage progression over time at both the patient and the individual joint level [Van Leeuwen *et al.* 1994; Smolen *et al.* 2006; Boers *et al.* 2001; Klarenbeek *et al.* 2010]. A proof of concept of the tight relationship between clinically active joints and structural changes is provided by the demonstration that repair (the opposite of progression), although it remains an extremely rare feature in RA [van der Linden *et al.* 2010], may only occur in association with improvement or cessation of clinical swelling [Lukas *et al.* 2010]. The crucial importance of joint assessment to predict radiographic outcomes in patients with RA is further highlighted by recent evidences showing that joint damage progression in remission is driven by residual swollen joints [Aletaha and Smolen, 2011], which appear to be more predictive compared with other variables of inflammation such as acute phase reactants [Aletaha *et al.* 2011]. The association between clinical inflammation and joint destruction has strong foundation in patients treated synthetic DMARDs. It is, however, worthwhile to underline that the relationship is not as clearly defined in patients treated with biologic agents, in which an apparent dissociation between clinical and radiographic outcomes can be observed. Patients with clinical disease activity treated with anti-TNF (tumor necrosis factor) agents may indeed have no evidence of erosive progression [Smolen *et al.* 2005]. Accordingly, SJCs averaged over time appear associated with radiographic outcomes during methotrexate monotherapy but not during combination therapy with infliximab [Smolen *et al.* 2006]. The fact that treatment choice *per se* is a major determinant of radiographic progression is further highlighted by recent evidence demonstrating that other predictors (autoantibody status, acute phase reactants, baseline erosion scores), alone or in combination, perform differently in patients receiving different treatment strategies [Visser *et al.* 2010].

If structural outcomes can collectively be determined by persistent joint swelling, a clinically relevant issue is whether the severity of joint

involvement at baseline may be equally predictive of the rate of radiographic progression over time. This would imply that patients with high levels of disease activity at presentation will continue to do badly. The relationship between the amount of initial joint inflammation and the persistence of inflammation itself, however, is not so obvious [Drouin and Haraoui, 2010]. Accordingly, there are conflicting results from the literature concerning the predictive value of a single assessment of SJCs at baseline for radiographic damage. In the long-term study by Kaarela [Kaarela, 1985], the number of swollen joints was an independent predictive factor of radiological progression. This association was either weaker [Smolen *et al.* 2006] or not confirmed in more recent studies [Combe *et al.* 2001; Machold *et al.* 2007; Courvoisier *et al.* 2008]. Such a discrepancy is also reflected by the variable inclusion of SJC in the recently developed matrix risk models for the prediction of rapid radiographic progression in RA [Vastesaeger *et al.* 2009; Visser *et al.* 2010]. In contrast, re-assessment of joint involvement soon after initiation of therapy seems more informative on radiographic outcomes [Smolen *et al.* 2006], in line with the notion that the strongest determinant of persistent disease activity is the level of disease activity itself after the first few months of treatment [Aletaha *et al.* 2007].

Imaging assessment

Modern ultrasonography (US) enables clinicians to sensitively assess soft-tissue structures, and the use of power Doppler (PD) further extends the analysis to synovial vascularity. Being more sensitive than clinical examination in detecting synovial inflammation [Naredo *et al.* 2005] and in discriminating between 'active' and 'inactive' joints, US-PD was expected to provide additional value compared with manual joint counts in predicting the evolution of RA. Using radiographic progression as a final measure of disease outcome, results have been however conflicting. If on the one hand time-integrated values of US-PD correlate well with the amount of joint damage progression [Naredo *et al.* 2007], a single time assessment at baseline has not been consistently associated with radiographic outcomes [Taylor *et al.* 2004; Naredo *et al.* 2007; Reynolds *et al.* 2009; Fukae *et al.* 2010]. Thus, similarly to SJCs, measuring cumulative synovial inflammation over time is more informative compared with single time point measurements, and highest levels of synovitis at baseline do not appear to predict *per se* worst disease evolution, irrespective of how 'sensitive' synovial inflammation is measured. Also, it is important to emphasize that the very high sensitivity of advanced PD equipments may to some extent compromise specificity. PD activity is indeed observed in the hand and finger joints of a number of healthy individuals [Terslev *et al.* 2008]. Similarly, US-PD measurements may be affected by physiological or pharmaceutical stimuli also in RA patients [Ellegaard *et al.* 2009; Zayat *et al.* 2011]. Altogether, these limitations should be taken into account when considering the prognostic value of US-PD assessment of disease activity. A particular setting in which US-PD may provide valuable information for disease prognostication is the state of clinical remission or low disease activity. Here indeed inflammatory findings on US-PD are commonly seen [Brown *et al.* 2006, 2008; Scirè *et al.* 2009], and both grayscale synovial hypertrophy and PD synovitis have been demonstrated to be independent predictors of structural progression on radiographs [Brown *et al.* 2008]. Owing to the lack of comparison between the predictive value of manual SJCs and US-PD scores in these patients, however, the superiority of imaging techniques compared with physical examination remains to be demonstrated.

Similarly to US-PD, synovitis detected through magnetic resonance imaging (MRI) does not appear predictive of radiographic erosions when assessed at baseline in active disease [McQueen *et al.* 2003; Bøyesen *et al.* 2011], again confirming the weak prognostic value of single time measures. Accordingly, a recent systematic review reported a variable ability of MRI findings to predict radiographic progression (range 18–100% for sensitivity and 5.9–97% for specificity), thus not recommending its routine clinical use [Suter *et al.* 2011]. MRI, however, can uniquely visualize another relevant inflammatory lesion in RA, the bone marrow edema (BME), which appears as an increased signal intensity on fat-suppressed T2-weighted, proton density or short tau inversion recovery (STIR) pulse sequences, consistent with increased water content [Østergaard *et al.* 2003]. Although BME is not specific for RA, its histopathological characterization in long-standing disease has consistently shown that BME truly corresponds to intramarrow inflammation [Jimenez-Boj *et al.* 2007; McQueen *et al.* 2007]. Differently from synovitis, BME appears as a strong predictor of radiographic progression in both early and established disease even

when single baseline assessments are performed [McQueen *et al.* 1999; Hetland *et al.* 2009, 2010; Bøyesen *et al.* 2011]. In particular, in the study by Hetland and colleagues, the predictive value of MRI was investigated in a standardized treatment setting against a number of other relevant prognostic factors, such as immunologic (ACPA, IgM RF, IgA RF), environmental (smoking, educational level), genetic (shared epitope) and disease activity markers [Hetland *et al.* 2009]. In this comprehensive model, MRI BME at presentation was the strongest independent predictor of radiographic progression 2 years later in early RA patients as determined in both multivariable linear and logistic regression analyses. The 5-year extension study in the same cohort confirmed the predictive ability of BME in the long term [Hetland *et al.* 2010]. The different prognostic significance of the assessment of BME at baseline compared with synovitis is currently unexplained. Whether BME represents a more sustained inflammatory process remains to be demonstrated, and different accessibility of the subchondral bone marrow compartment to treatment and/or different cellular composition of the inflammatory infiltrates are only speculative possibilities.

Synovial tissue analysis

Histological and molecular analyses of the synovial membrane ideally represent the gold standard for the assessment of joint inflammation in RA. The synovial tissue indeed undergoes early inflammatory changes, which include resident cell activation and infiltration by hematopoietic cells [Hitchon and El-Gabalawy, 2011], that can be clearly recognized even when clinical signs of arthritis are lacking [Kraan *et al.* 1998]. Compared with clinical and imaging assessments, examination of the synovial tissue offers the potential advantage of capturing both quantitative and qualitative features of the inflammatory process of RA. The rheumatoid lesion is indeed characterized by a high degree of cellular and molecular heterogeneity across different disease phases and clinical subsets [Bugatti *et al.* 2011a]. The pathophysiological background and the clinical correlates of such heterogeneity have only in part started to be understood. Synovial tissue analysis is therefore at present mainly a research area, with limited clinical value in daily practice. However, as synovitis is the primary event underlying signs and symptoms of arthritis in RA, increasing efforts are being made in order to identify synovial prognostic biomarkers that could be used in individual patients [de Hair *et al.* 2011]. The widespread diffusion of mini-invasive techniques for synovial sampling has recently made the clinical use of synovial tissue analysis a more realistic goal. Major drawbacks that need to be considered in light of the potential for clinical translation include the fact that synovial biopsy, although 'mini', remains an invasive procedure and requires some technical skills and facilities that might ultimately influence the outcome. In addition, the synovial tissue is more easily accessible in larger joints such as the knee, which is generally affected in patients with a more aggressive disease course. This could account for selection bias. Finally, although multiple sampling from different locations within the joint is currently recommended [van de Sande *et al.* 2011], overestimation or underestimation of inflammation due to the wide heterogeneity of the synovium cannot be excluded completely.

A number of independent studies have tried to identify the microscopic and molecular factors possibly associated with joint damage progression in RA. One of the cell populations which has historically attracted great interest are synovial marcophages. It is widely accepted that the number of CD68+ macrophages in the sublining strongly correlates with local disease activity [Tak *et al.* 1997] and systemic inflammation [Baeten *et al.* 2005], and is sensitive to change following active antirheumatic therapies [Haringman *et al.* 2005]. The dynamic correlation of tissue macrophages with the levels of ongoing inflammation, however, intrinsically limits their possible long-term prognostic value as a single time measure. The association between the degree of synovial macrophage infiltration and the development of bone erosions at follow up appears indeed highly controversial [Mulherin *et al.* 1996; Kraan *et al.* 2004]. The same applies to macrophage-derived cytokines, none of which has been shown to accurately predict joint damage progression in RA [Rooney *et al.* 2010].

Another relevant aspect of synovial tissue heterogeneity with possible pathophysiologic and clinical value is the degree and local organization of infiltrating T and B lymphocytes, which have been classically described as either randomly distributed within the sublining or spatially grouped into discrete lymphoid microenvironments [Takemura *et al.* 2001]. The functional properties associated with the development of T–B cell niches in the context of the chronically

inflamed synovium are beyond the scope of this review and have been extensively summarized elsewhere [Manzo *et al.* 2010]. Different lymphoid patterns are also been intensively investigated for their possible translational value. Depending on the histological criteria adopted to identify lymphocytic aggregates as well as the cutoff values set for defining lymphoid aggregation, different clinical studies yielded partly conflicting conclusions on whether these structures are associated with more severe RA subsets in terms of radiographic damage [Klimiuk *et al.* 2003; Thurlings *et al.* 2008; Cañete *et al.* 2009]. However, it appears that the more selectively we look at specific B-cell-centered processes the more unfavorable outcomes we capture. When B cells are analyzed as core elements of the process of lymphocytic aggregation, indeed, increasing degrees of B-cell infiltration appear to be associated with a higher prevalence of erosive disease cross-sectionally, in parallel with an unbalanced molecular milieu favoring bone remodeling [Bugatti *et al.* 2011b]. These same findings are true when other cell populations of the B-cell lineage are analyzed, such as CD79a+ B cells [Mo *et al.* 2011]. Whether B-cell-rich synovitis and bone erosions are parallel manifestations of a more severe disease subset or are temporarily distinct phenomena remains to be demonstrated. Furthermore, as B cells are precursors of antibody-producing cells and autoantibodies are recognized as relevant prognostic factors in erosive RA, the clinical value of synovial B-cell infiltration theoretically may not be independent from the autoantibody status. The relationship between synovial lymphoid aggregates and RA-specific autoantibodies is however not unequivocal. Whilst previous studies have suggested that the process of lymphocytic aggregation is neither restricted nor preferentially associated with autoantibody positivity [Thurlings *et al.* 2008; Cantaert *et al.* 2008], recent data obtained in murine chimeric systems demonstrated a significant relationship between synovial B-cell niches and ongoing production of ACPA [Humby *et al.* 2009]. Overall, the independent prognostic value of synovial B-cell infiltration is at present nondeterminable, and prospective studies in early RA are awaited.

Synovial tissue biomarkers in peripheral blood

Overall, the specificity of clinical and imaging assessments of inflammation at disease onset is too low to make such variables fully predictive of joint damage progression in RA. On the other hand, synovial tissue analysis is still in its infancy in terms of clinical translation. Great efforts are thus being made in order to identify novel variables which reliably reflect the pathophysiological cascade of RA and which can be used as prognostic markers of disease severity. In this field, serological biomarkers reflecting bone and cartilage destruction are emerging as valid tools and have been the subject of a recent review [Karsdal *et al.* 2011]. As bone and cartilage turnover represent the downstream effect of joint inflammation, equal attention is in parallel being paid to possible biomarkers of synovitis.

C-reactive protein (CRP) and ESR are only indirectly linked to synovitis. The acute phase proteins are indeed primarily produced by hepatocytes in response to interleukins (IL) released during inflammation [Gabay and Kushner, 1999]. Although the predictive value of ESR and, to a lesser extent, of CRP for radiographic progression has been proposed in several independent studies [Combe *et al.* 2001; Lindqvist *et al.* 2003, 2005], their value compared with physical examination appears overall poor [Aletaha *et al.* 2011]. Disappointingly, however, none of the cytokines that drive the acute systemic response and that can be produced at least in part at the synovial tissue level, such as IL-1, IL-6 and TNF-α, has been convincingly shown to be associated with progression of joint damage [Roux-Lombard *et al.* 2001; Rooney *et al.* 2010], thus making the crude assessment of classical inflammatory biomarkers hardly advisable.

Additional value could be provided by serological markers which reflect more specific aspects of synovial pathology, differently regulated in different patients, such as angiogenesis (and vascular biology in general) and mononuclear cell infiltration. The degree of activation of the vascular endothelium, as assessed through E-selectin serum levels, has been shown to be associated with progression of joint destruction in early RA [Kuuliala *et al.* 2002], and similar data have been also provided for vascular endothelial growth factor (VEGF) [Ballara *et al.* 2001]. More recently, markers of different T-cell subsets, such as granzyme B and IL-22, have been shown to be elevated in the serum of RA patients in association with radiographic progression [Goldbach-Mansky *et al.* 2005; Leipe *et al.* 2011]. Overall, the specificity and predictive value of such

markers would however need confirmation in further studies.

An emerging peripheral biomarker of synovitis is the chemokine CXCL13. CXCL13 is involved in the positioning, cooperation and activation of B and T cells within lymphoid and extralymphoid sites, including RA synovium [Loetscher and Moser, 2002; Manzo *et al.* 2010]. Peripheral blood and serum expression of CXCL13 are increased in RA patients compared with healthy donors [Manzo *et al.* 2008; Rioja *et al.* 2008; Rosengren *et al.* 2011], and preliminary results indicate a significant correlation between serum and synovial tissue levels of CXCL13 [Rosengren *et al.* 2011]. CXCL13 appears as a marker of severity in RA. A large prospective study has indeed shown that early RA patients with the highest levels of serum CXCL13 are those with the highest rate of progression of joint damage over long-term follow up [Meeuwisse *et al.* 2011]. In line with these data, we could demonstrate that serum levels of CXCL13 in untreated patients with disease duration <12 months are associated with clinical and US synovitis and predict US-PD signal persistence [Bugatti *et al.* 2012]. Importantly, both in the study by Meeuwisse and colleagues and in our previous study, the predictive value of CXCL13 was independent of the level of inflammation indicated by the CRP level as well as the ACPA status [Meeuwisse *et al.* 2011; Bugatti *et al.* 2012]. Thus, although larger, prospective, confirmatory studies are needed, there may be opportunities to identify peripheral blood biomarkers capable of reflecting synovial pathology and predicting clinical outcomes in RA.

Conclusions

Patients with different outcomes are still difficult to identify in the earliest stages of the disease, and RA phenotypic heterogeneity remains largely unpredictable. As joint damage progression is the result of the cumulative burden of inflammation over time, the identification of disease variables associated with persistent, refractory joint inflammation could provide valuable tools to determine which patient is to run a more severe and rapid radiographic course. Joint inflammation can now be assessed through a variety of different techniques able to provide increasing information on tissue pathobiology. Achievements in the field of synovial tissue analysis and biomarkers of synovitis in the peripheral blood represent only the first steps of the ongoing progress and cannot currently guide clinical decision making. Further research is needed to integrate clinical, imaging and biologic studies into comprehensive models able to accurately predict disease severity and stratify patients into prognostic subgroups.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

Aletaha, D., Alasti, F. and Smolen, J. (2011) Rheumatoid arthritis near remission: clinical rather than laboratory inflammation is associated with radiographic progression. *Ann Rheum Dis* 70: 1975–1980.

Aletaha, D., Funovits, J., Keystone, E. and Smolen, J. (2007) Disease activity early in the course of treatment predicts response to therapy after one year in rheumatoid arthritis patients. *Arthritis Rheum* 56: 3226–3235.

Aletaha, D. and Smolen, J. (2011) Joint damage in rheumatoid arthritis progresses in remission according to the Disease Activity Score in 28 joints and is driven by residual swollen joints. *Arthritis Rheum* 63: 3702–3711.

Baeten, D., Kruithof, E., De Rycke, L., Boots, A., Mielants, H., Veys, E. *et al.* (2005) Infiltration of the synovial membrane with macrophage subsets and polymorphonuclear cells reflects global disease activity in spondyloarthropathy. *Arthritis Res Ther* 7: R359–R369.

Ballara, S., Taylor, P., Reusch, P., Marmé, D., Feldmann, M., Maini, R. *et al.* (2001) Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. *Arthritis Rheum* 44: 2055–2064.

Boers, M., Kostense, P., Verhoeven, A. and van der Linden, S. for the COBRA Trial Group (2001) Inflammation and damage in an individual joint predict further damage in that joint in patients with early rheumatoid arthritis. *Arthritis Rheum* 44: 2242–2246.

Bøyesen, P., Haavardsholm, E., Ostergaard, M., van der Heijde, D., Sesseng, S. and Kvien, T. (2011) MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 70: 428–433.

Brown, A., Conaghan, P., Karim, Z., Quinn, M., Ikeda, K., Peterfy, C. *et al.* (2008) An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* 58: 2958–2967.

Brown, A., Quinn, M., Karim, Z., Conaghan, P., Peterfy, C., Hensor, E. *et al.* (2006) Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression. *Arthritis Rheum* 54: 3761–3773.

Bugatti, S., Manzo, A., Benaglio, F., Klersy, C., Vitolo, B., Todoerti, M. *et al.* (2012) Serum levels of CXCL13 are associated with ultrasonographic synovitis and predict power Doppler persistence in early rheumatoid arthritis treated with non-biological disease-modifying anti-rheumatic drugs. *Arthritis Res Ther* 14: R34.

Bugatti, S., Manzo, A., Bombardieri, M., Vitolo, B., Humby, F., Kelly, S. *et al.* (2011a) Synovial tissue heterogeneity and peripheral blood biomarkers. *Curr Rheumatol Rep* 13: 440–448.

Bugatti, S., Manzo, A., Vitolo, B., Fusetti, C., Humby, F., Caporali, R. *et al.* (2011b) B cell distribution and activation-induced cytidine deaminase expression in rheumatoid synovitis: clinical and bio-molecular correlates. *Ann Rheum Dis* 70: A55.

Cañete, J., Celis, R., Moll, C., Izquierdo, E., Marsal, S., Sanmartí, R. *et al.* (2009) Clinical significance of synovial lymphoid neogenesis and its reversal after anti-tumour necrosis factor alpha therapy in rheumatoid arthritis. *Ann Rheum Dis* 68: 751–756.

Cantaert, T., Kolln, J., Timmer, T., van der Pouw Kraan, T., Vandooren, B., Thurlings, R. *et al.* (2008) B lymphocyte autoimmunity in rheumatoid synovitis is independent of ectopic lymphoid neogenesis. *J Immunol* 181: 785–794.

Combe, B., Dougados, M., Goupille, P., Cantagrel, A., Eliaou, J., Sibilia, J. *et al.* (2001) Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. *Arthritis Rheum* 44: 1736–1743.

Courvoisier, N., Dougados, M., Cantagrel, A., Goupille, P., Meyer, O., Sibilia, J. *et al.* (2008) Prognostic factors of 10-year radiographic outcome in early rheumatoid arthritis: a prospective study. *Arthritis Res Ther* 10:R106.

de Hair, M., Harty, L., Gerlag, D., Pitzalis, C., Veale, D. and Tak, P. (2011) Synovial tissue analysis for the discovery of diagnostic and prognostic biomarkers in patients with early arthritis. *J Rheumatol* 38: 2068–2072.

Drouin, J. and Haraoui, B. for the 3e Initiative Group (2010) Predictors of clinical response and radiographic progression in patients with rheumatoid arthritis treated with methotrexate monotherapy. *J Rheumatol* 37: 1405–1410.

Drossaers-Bakker, K., de Buck, M., van Zeben, D., Zwinderman, A., Breedveld, F. and Hazes, J. (1999) Long-term course and outcome of functional capacity in rheumatoid arthritis: the effect of disease activity and radiologic damage over time. *Arthritis Rheum* 42: 1854–1860.

Ellegaard, K., Torp-Pedersen, S., Henriksen, M., Lund, H., Danneskiold-Samsøe, B. and Bliddal, H. (2009) Influence of recent exercise and skin temperature on ultrasound Doppler measurements in patients with rheumatoid arthritis - an intervention study. *Rheumatology* 48: 1520–1523.

Forslind, K., Ahlmén, M., Eberhardt, K., Hafström, I. and Svensson, B. for the BARFOT Study Group (2004) Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 63: 1090–1095.

Fukae, J., Kon, Y., Henmi, M., Sakamoto, F., Narita, A., Shimizu, M. *et al.* (2010) Change of synovial vascularity in a single finger joint assessed by power Doppler sonography correlated with radiographic change in rheumatoid arthritis: comparative study of a novel quantitative score with a semiquantitative score. *Arthritis Care Res* 62: 657–663.

Gabay, C. and Kushner, I. (1999) Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340: 448–454.

Goldbach-Mansky, R., Suson, S., Wesley, R., Hack, C., El-Gabalawy, H. and Tak, P. (2005) Raised granzyme B levels are associated with erosions in patients with early rheumatoid factor positive rheumatoid arthritis. *Ann Rheum Dis* 64: 715–721.

Haringman, J., Gerlag, D., Zwinderman, A., Smeets, T., Kraan, M., Baeten, D. *et al.* (2005) Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 64: 834–838.

Hetland, M., Ejbjerg, B., Hørslev-Petersen, K., Jacobsen, S., Vestergaard, A., Jurik, A. *et al.* (2009) MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 68: 384–390.

Hetland, M., Stengaard-Pedersen, K., Junker, P., Østergaard, M., Ejbjerg, B., Jacobsen, S. *et al.* (2010) Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the

5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 69: 1789–1795.

Hitchon, C. and El-Gabalawy, H. (2011) The synovium in rheumatoid arthritis. *Open Rheumatol J* 5: 107–114.

Humby, F., Bombardieri, M., Manzo, A., Kelly, S., Blades, M., Kirkham, B. *et al.* (2009) Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med* 6: e1.

Jimenez-Boj, E., Nöbauer-Huhmann, I., Hanslik-Schnabel, B., Dorotka, R., Wanivenhaus, A., Kainberger, F. *et al.* (2007) Bone erosions and bone marrow edema as defined by magnetic resonance imaging reflect true bone marrow inflammation in rheumatoid arthritis. *Arthritis Rheum* 56: 1118–1124.

Kaarela, K. (1985) Prognostic factors and diagnostic criteria in early rheumatoid arthritis. *Scand J Rheumatol Suppl* 57: 1–54.

Karsdal, M., Woodworth, T., Henriksen, K., Maksymowych, W., Genant, H., Vergnaud, P. *et al.* (2011) Biochemical markers of ongoing joint damage in rheumatoid arthritis—current and future applications, limitations and opportunities. *Arthritis Res Ther* 13: 215.

Klarenbeek, N., Güler-Yüksel, M., van der Heijde, D., Hulsmans, H., Kerstens, P., Molenaar, T. *et al.* (2010) Clinical synovitis in a particular joint is associated with progression of erosions and joint space narrowing in that same joint, but not in patients initially treated with infliximab. *Ann Rheum Dis* 69: 2107–2113.

Klimiuk, P., Sierakowski, S., Latosiewicz, R., Cylwik, J., Cylwik, B., Skowronski, J. *et al.* (2003) Circulating tumour necrosis factor alpha and soluble tumour necrosis factor receptors in patients with different patterns of rheumatoid synovitis. *Ann Rheum Dis* 62: 472–475.

Kraan, M., Haringman, J., Weedon, H., Barg, E., Smith, M., Ahern, M. *et al.* (2004) 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* 63:483–488.

Kraan, M., Versendaal, H., Jonker, M., Bresnihan, B., Post, W., t Hart, B. *et al.* (1998) Asymptomatic synovitis precedes clinically manifest arthritis. *Arthritis Rheum* 41: 1481–148.

Kuuliala, A., Eberhardt, K., Takala, A., Kautiainen, H., Repo, H. and Leirisalo-Repo, M. (2002) Circulating soluble E-selectin in early rheumatoid arthritis: a prospective five year study. *Ann Rheum Dis* 61: 242–246.

Leipe, J., Schramm, M., Grunke, M., Baeuerle, M., Dechant, C., Nigg, A. *et al.* (2011) Interleukin 22 serum levels are associated with radiographic progression in rheumatoid arthritis. *Ann Rheum Dis* 70: 1453–1457.

Liao, K., Weinblatt, M., Cui, J., Iannaccone, C., Chibnik, L., Lu, B. *et al.* (2011) Clinical predictors of erosion-free status in rheumatoid arthritis: a prospective cohort study. *Rheumatology* 50: 1473–1479.

Lillegraven, S., van der Heijde, D., Uhlig, T., Kvien, T. and Haavardsholm, E. (2012) What is the clinical relevance of erosions and joint space narrowing in RA? *Nat Rev Rheumatol*, DOI: 10.1038/nrrheum.2011.20210.1038/nrrheum.2011.202.

Lindqvist, E., Eberhardt, K., Bendtzen, K., Heinegård, D. and Saxne, T. (2005) Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 64: 196–201.

Lindqvist, E., Jonsson, K., Saxne, T. and Eberhardt, K. (2003) Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. *Ann Rheum Dis* 62: 611–616.

Loetscher, P. and Moser, B. (2002). Homing chemokines in rheumatoid arthritis. *Arthritis Res* 4: 233–236.

Lukas, C., van der Heijde, D., Fatenajad, S. and Landewé, R. (2010) Repair of erosions occurs almost exclusively in damaged joints without swelling. *Ann Rheum Dis* 69: 851–855.

Machold, K., Stamm, T., Nell, V., Pflugbeil, S., Aletaha, D., Steiner, G. *et al.* (2007) Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. *Rheumatology* 46: 342–349.

Manzo, A., Bombardieri, M., Humby, F. and Pitzalis, C. (2010) Secondary and ectopic lymphoid tissue responses in rheumatoid arthritis: from inflammation to autoimmunity and tissue damage/remodeling. *Immunol Rev* 233: 267–285.

Manzo, A., Vitolo, B., Humby, F., Caporali, R., Jarrossay, D., Dell'accio, F. *et al.* (2008) Mature antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13 in the inflammatory environment of the rheumatoid joint. *Arthritis Rheum* 58: 3377–3387.

Markatseli, T., Papagoras, C. and Drosos, A. (2010) Prognostic factors for erosive rheumatoid arthritis. *Clin Exp Rheumatol* 28: 114–123.

McQueen, F., Benton, N., Perry, D., Crabbe, J., Robinson, E., Yeoman, S. *et al.* (2003) Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 48: 1814–1827.

McQueen, F., Gao, A., Ostergaard, M., King, A., Shalley, G., Robinson, E. *et al.* (2007) High-grade MRI bone oedema is common within the surgical field in rheumatoid arthritis patients undergoing joint replacement and is associated with osteitis in subchondral bone. *Ann Rheum Dis* 66: 1581–1587.

McQueen, F., Stewart, N., Crabbe, J., Robinson, E., Yeoman, S., Tan, P. *et al.* (1999) Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 58: 156–163.

Meeuwisse, C., van der Linden, M., Rullmann, T., Allaart, C., Nelissen, R., Huizinga, T. *et al.* (2011) Identification of CXCL13 as a marker for rheumatoid arthritis outcome using an in silico model of the rheumatic joint. *Arthritis Rheum* 63: 1265–1273.

Meyer, O., Labarre, C., Dougados, M., Goupille, P., Cantagrel, A., Dubois, A. *et al.* (2003) Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 62: 120–126.

Mo, Y., Dai, L., Zheng, D., Zhu, L., Wei, X., Pessler, F. *et al.* (2011) Synovial infiltration with CD79a-positive B cells, but not other B cell lineage markers, correlates with joint destruction in rheumatoid arthritis. *J Rheumatol* 38: 2301–2308.

Mulherin, D., Fitzgerald, O. and Bresnihan, B. (1996) Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. *Arthritis Rheum* 39: 115–124.

Naredo, E., Bonilla, G., Gamero, F., Uson, J., Carmona, L. and Laffon, A. (2005) Assessment of inflammatory activity in rheumatoid arthritis: a comparative study of clinical evaluation with grey scale and power Doppler ultrasonography. *Ann Rheum Dis* 64: 375–381.

Naredo, E., Collado, P., Cruz, A., Palop, M., Cabero, F., Richi, P. *et al.* (2007) Longitudinal power Doppler ultrasonographic assessment of joint inflammatory activity in early rheumatoid arthritis: predictive value in disease activity and radiologic progression. *Arthritis Rheum* 57: 116–124.

Nell, V., Machold, K., Eberl, G., Stamm, T., Uffmann, M. and Smolen, J. (2004) Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. *Rheumatology* 43: 906–914.

Østergaard, M., Peterfy, C., Conaghan, P., McQueen, F., Bird, P., Ejbjerg, B. *et al.* (2003) OMERACT Rheumatoid Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. *J Rheumatol* 30: 1385–1386.

Reynolds, P., Heron, C., Pilcher, J. and Kiely, P. (2009) Prediction of erosion progression using ultrasound in established rheumatoid arthritis: a 2-year follow-up study. *Skeletal Radiol* 38: 473–478.

Rezaei, H., Saevarsdottir, S., Forslind, K., Albertsson, K., Wallin, H., Bratt, J. *et al.* (2012) In early rheumatoid arthritis, patients with a good initial response to methotrexate have excellent 2-year clinical outcomes, but radiological progression is not fully prevented: data from the methotrexate responders population in the SWEFOT trial. *Ann Rheum Dis* 71: 186–191.

Rioja, I., Hughes, F., Sharp, C., Warnock, L., Montgomery, D., Akil, M. *et al.* (2008) Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL13, CCL23, transforming growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. *Arthritis Rheum* 58: 2257–2267.

Rooney, T., Roux-Lombard, P., Veale, D., FitzGerald, O., Dayer, J. and Bresnihan, B. (2010) Synovial tissue and serum biomarkers of disease activity, therapeutic response and radiographic progression: analysis of a proof-of-concept randomised clinical trial of cytokine blockade. *Ann Rheum Dis* 69: 706–714.

Rosengren, S., Wei, N., Kalunian, K., Kavanaugh, A. and Boyle, D. (2011) CXCL13: a novel biomarker of B-cell return following rituximab treatment and synovitis in patients with rheumatoid arthritis. *Rheumatology* 50: 603–610.

Roux-Lombard, P., Eberhardt, K., Saxne, T., Dayer, J. and Wollheim, F. (2001) Cytokines, metalloproteinases, their inhibitors and cartilage oligomeric matrix protein: relationship to radiological progression and inflammation in early rheumatoid arthritis. A prospective 5-year study. *Rheumatology* 40: 544–551.

Schett, G. (2007) Joint remodelling in inflammatory disease. *Ann Rheum Dis* 66(Suppl. 3): iii42–iii44.

Scirè, C., Montecucco, C., Codullo, V., Epis, O., Todoerti, M. and Caporali, R. (2009) Ultrasonographic evaluation of joint involvement in early rheumatoid arthritis in clinical remission: power Doppler signal predicts short-term relapse. *Rheumatology* 48: 1092–1097.

Scott, D., Pugner, K., Kaarela, K., Doyle, D., Woolf, A., Holmes, J. *et al.* (2000) The links between joint damage and disability in rheumatoid arthritis. *Rheumatology* 39: 122–132.

Smolen, J., Han, C., Bala, M., Maini, R., Kalden, J., van der Heijde, D. *et al.* (2005) Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had

no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. *Arthritis Rheum* 52: 1020–1030.

Smolen, J., van der Heijde, D., St.Clair, E., Emery, P., Bathon, J., Keystone, E. *et al.* for the Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis of Early Onset (ASPIRE) Study Group (2006) Predictors of joint damage in patients with early rheumatoid arthritis treated with highdose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. *Arthritis Rheum* 54: 702–710.

Suter, L., Fraenkel, L. and Braithwaite, R. (2011) Role of magnetic resonance imaging in the diagnosis and prognosis of rheumatoid arthritis. *Arthritis Care Res* 63: 675–688.

Syversen, S., Gaarder, P., Goll, G., Ødegård, S., Haavardsholm, E., Mowinckel, P. *et al.* (2008) High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 67: 212–217.

Tak, P., Smeets, T., Daha, M., Kluin, P., Meijers, K., Brand, R. *et al.* (1997) Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum* 40: 217–225.

Takemura, S., Braun, A., Crowson, C., Kurtin, P., Cofield, R., O'Fallon, W. *et al.* (2001) Lymphoid neogenesis in rheumatoid synovitis. *J Immunol* 167: 1072–1080.

Taylor, P., Steuer, A., Gruber, J., Cosgrove, D., Blomley, M., Marsters, P. *et al.* (2004) Comparison of ultrasonographic assessment of synovitis and joint vascularity with radiographic evaluation in a randomized, placebo-controlled study of infliximab therapy in early rheumatoid arthritis. *Arthritis Rheum* 50: 1107–1116.

Terslev, L., von der Recke, P., Torp-Pedersen, S., Koenig, M. and Bliddal, H. (2008) Diagnostic sensitivity and specificity of Doppler ultrasound in rheumatoid arthritis. *J Rheumatol* 35: 49–53.

Thurlings, R., Wijbrandts, C., Mebius, R., Cantaert, T., Dinant, H., van der Pouw-Kraan, T. *et al.* (2008) Synovial lymphoid neogenesis does not define a specific clinical rheumatoid arthritis phenotype. *Arthritis Rheum* 58: 1582–1589.

van de Sande, M., Gerlag, D., Lodde, B., van Baarsen, L., Alivernini, S., Codullo, V. *et al.* (2011) Evaluating antirheumatic treatments using synovial biopsy: a recommendation for standardisation to be used in clinical trials. *Ann Rheum Dis* 70: 423–427.

van der Helm-van Mil, A., Verpoort, K., Breedveld, F., Toes, R. and Huizinga, T. (2005) Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 7: R949–R958.

van der Linden, M., Boja, R., Klarenbeek, N., Huizinga, T., van der Heijde, D. and van der Helm-van Mil, A. (2010) Repair of joint erosions in rheumatoid arthritis: prevalence and patient characteristics in a large inception cohort. *Ann Rheum Dis* 69: 727–729.

Van Leeuwen, M., van der Heijde, D., van Rijswijk, M., Houtman, P., van Riel, P., van de Putte, L. *et al.* (1994) Interrelationship of outcome measures and process variables in early rheumatoid arthritis: a comparison of radiologic damage, physical disability, joint counts, and acute phase reactants. *J Rheumatol* 21: 425–429.

Vastesaeger, N., Xu, S., Aletaha, D., St Clair, E. and Smolen, J. (2009) A pilot risk model for the prediction of rapid radiographic progression in rheumatoid arthritis. *Rheumatology* 48: 1114–1121.

Visser, K., Goekoop-Ruiterman, Y., de Vries-Bouwstra, J., Ronday, H., Seys, P., Kerstens, P. *et al.* (2010) A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the BeSt study. *Ann Rheum Dis* 69: 1333–1337.

Walsh, N. and Gravallese, E. (2010) Bone remodeling in rheumatic disease: a question of balance. *Immunol Rev* 233: 301–312.

Welsing, P., van Gestel, A., Swinkels, H., Kiemeney, L. and van Riel, P. (2001) The relationship between disease activity, joint destruction, and functional capacity over the course of rheumatoid arthritis. *Arthritis Rheum* 44: 2009–2017.

Zayat, A., Conaghan, P., Sharif, M., Freeston, J., Wenham, C., Hensor, E. *et al.* (2011) Do non-steroidal anti-inflammatory drugs have a significant effect on detection and grading of ultrasound-detected synovitis in patients with rheumatoid arthritis? Results from a randomised study. *Ann Rheum Dis* 70: 1746–1751.