Review

The potential roles for novel biomarkers in rheumatoid arthritis assessment

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ABSTRACT
Comprehensive management of rheumatoid arthritis (RA) requires regular monitoring of disease activity, functional status, and structural damage to facilitate optimal patient outcomes. Tight control strategies have been successfully used in other diseases including diabetes and hypertension. Tight control requires frequent disease activity measurements in order to tailor treatment for individual patients, resulting in improved patient outcomes. Current monitoring measures used in clinical practice are largely driven by subjective evaluation of signs and symptoms, which are critical but limited by assessor variability and may not reflect true biological change in a timely manner. Research suggests that novel biomarkers may provide quantitative, objective assessments of disease activity and structural damage risk in RA, which are not captured by current measures. The simultaneous use of multiple biomarkers in a single test algorithm may provide a more comprehensive quantitative representation of the overall complex heterogeneous biology of RA. This article reviews the current management strategies for monitoring RA and the potential impact that multi-biomarker assays may have on RA assessment, which may further improve clinical outcomes.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, with 1% to 2% worldwide prevalence (1). Although the incidence of RA varies geographically, the hallmarks of this progressive disease are the same for all patients – inflammation, joint pain, and bone and cartilage destruction that can lead to functional disability (2). The last decade witnessed significant advances in the treatment of RA, including improved treatment strategies and the introduction of new therapies, both traditional disease-modifying antirheumatic drugs (DMARDs) and biologic response modifiers that target key inflammatory mediators of inflammation (3-5). Despite these treatment advances however, many patients continue to experience chronic inflammation, progressive joint damage, and disability. Only modest improvements in survival rates have been observed for the first time in the last 40 years (6, 7).

In addition to better therapeutic agents, "tight control" or "treat to target" strategies for RA management bring an additional option for improving outcomes – one which is focused on more quantitative and frequent disease activity assessment. According to these new management strategies, RA care should include regular quantitative monitoring of disease activity, functional status, and structural damage to facilitate timely and appropriate changes in treatment, resulting in optimal patient outcomes (8). The objectives of this review are to discuss the strengths and limitations of current assessment tools for these domains and to explore new paradigms. Specifically, this review will focus on biomarker-based assays and their potential to improve assessment of both disease activity and structural damage, which may thereby enhance clinical decision-making to improve patient outcomes.

Monitoring disease activity and structural damage in RA

Tight control strategies involve frequent disease activity measurements to tailor treatment decisions for individual patients with the goal of achieving a predefined level of remission or low disease activity (9, 10). Similar strategies have previously demonstrated improved patient outcomes in other disorders, such as diabetes (11, 12). In

RA, tight control approaches have been shown to improve patient outcomes in several clinical studies, including the FIN-RACo trial (13), the TiCoRA trial (14), the BeSt trial (15), and the CAMERA trial (16). (Table I) These studies uniformly demonstrate that regular, quantitative disease activity measurements guiding aggressive treatment changes can improve clinical and radiologic outcomes.

Recently published guidelines from the European League Against Rheumatism (EULAR) (17), a joint international taskforce entitled Treat to Target (10) and a joint EULAR/American College of Rheumatology (ACR) Committee (18) recommend that, in addition to disease activity, physicians should also monitor structural damage, including joint space narrowing, and erosions. The EULAR/ACR collaborative guidelines for reporting disease activity in clinical trials also recommend that disease activity, function, and damage domains are all monitored (18). Detecting and treating the earliest signs of structural damage can prevent disability and significantly improve long-term patient outcomes (19-21). Since patterns and rates of structural damage vary throughout the course of a patient's disease and do not always track with inflammation and clinical symptoms, monitoring skeletal damage in addition to disease activity remains critical for optimal management of RA.

Although disease activity and structural damage are linked, they are separable processes (22, 23). For example, erosions can continue even in the absence of clinical signs and symptoms when patients appear to be in clinical remission or low disease activity states. In a prospective study of 191 patients, Cohen et al. (24) analysed radiographs of hands, wrists, and feet, and found that 33% of RA patients in sustained remission at 3 and 5 years had a significant increase in radiographic damage between baseline and 5 years. Erosions were found in 36.7%, 53.3%, and 53.3% of patients in remission at baseline, 3 years, and 5 years, respectively. Other studies have also shown that despite the improvements obtained in clinical symptoms and inflammation with DMARD treatment, patients in clinical remission are still at risk for erosions (25, 26). Further evidence supports that the elevated inflammation associated with high disease activity states is not necessarily accompanied by severe erosive activity in all patients. For instance, patients without improvement of signs and symptoms on infliximab and methotrexate showed considerable benefit with regard to the joint destructive process, suggesting that inflammation and structural damage can be dissociated pathological processes (27).

Current disease-monitoring tools for RA

Clinical studies demonstrating improved outcomes with tight control typically used the Disease Activity Score 28 (DAS28) to measure disease activity. The DAS28 was developed and validated using a systematic process involving multiple independent studies (28). Although many RA clinical trials use the DAS28 to assess/measure disease activity, it has several limitations. First, several components of the DAS28, especially the swollen and tender joint counts and the patient global assessment, are subject to inter- and intra-observer variability (29-33). For example, a recent study of swollen joint counts confirmed that clinical inter- and intra-observer variability can be significant, and can have a substantial impact on the DAS28 score and consequently the management of patients (34). Second, calculation of a patient's DAS28 score can be time-consuming and cumbersome, as evidenced by the nominal use of DAS28 in community practice in the United States (35, 36).

There are a number of clinical assessment tools for measuring disease activity in RA besides the DAS28. Newer disease activity indices, such as the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI), were validated based on their correlation to DAS28 (27,37). The ACR Core Set can be used to determine the relative change in disease activity without determining the absolute level of disease activity, and is commonly used in clinical trials in the United States but (like the DAS28)

Table I. Characteristics of the tight control RA studies.

Study	Interventions/Groups	n.	Medications at the start	Frequency of assessment	Disease duration
FIN-RACo (13)	Combination therapy*	97	SSZ, MTX, HCQ ± Predn	3 months (variable)	<2 years
	Monotherapy	98	SSZ ± Predn	3/6 months (clinical decision/ variable)	<2 years
TICORA (14)	Intensive management*	55	DMARD, ia steroid	1 month (DAS)	<5 years
	Routine management	55	DMARD monotherapy	3 months (clinical decision)	<5 years
BeSt (15)	Sequential monotherapy*	126	MTX	3 months (DAS44)	<2 years
	Step-up combination therapy*	121	MTX	3 months (DAS44)	<2 years
	Initial combination therapy + hd predn*	133	MTX, SSZ, predn	3 months (DAS44)	<2 years
	Initial combination therapy + infliximab*	128	MTX, infliximab	3 months (DAS44)	<2 years
CAMERA (16)	Intensive strategy*	151	MTX	1 month (computer decision)	<1 year
	Conventional strategy	148	MTX	3 months (clinical decision)	<1 year

*Based on tight control treatment. BeSt; Behandel strategieen; CAMERA: Computer Assisted Management of Early Rheumatoid Arthritis; DAS: disease activity score; DMARD: disease-modifying antirheumatic drug; FIN-RACo: Finnish Rheumatoid Arthritis Combination Therapy; HCQ: hydrochloroquine; hd: high dose; ia: intra-articular; MTX: methotrexate; predn: prednisone; SSZ: sulfasalazine; TICORA: Tight Control of Rheumatoid Arthritis.

is not often used in clinical practice (38). These tools (*i.e.* the SDAI, CDAI, and ACR Core Set) are also based on swollen and tender joint counts and patient or physician global assessments, so, while again valuable, they do not eliminate the variability inherent in the DAS28 (39-45).

Finally, patient-reported outcome measures, such as the Rheumatology Assessment Patient Index Data (RAPID), use selected components of the multidimensional Health Assessment Questionnaire and focus on the patient's assessment of pain, fatigue, and functional status. These are critical assessments which guide care; however, they are also subjective and therefore inherently variable, both between patients and even within a patient, as the patient experiences the disease over time (36, 46).

Today, there are no validated laboratory tests that encompass the complex biological basis of RA. Standardised testing of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) allows quantitation of one aspect of RA disease activity. However, these are proteins associated with the acute phase response pathway. The pathophysiology driving RA inflammation is complex and heterogeneous, and CRP and ESR cannot capture the full complement of RA-specific disease processes, leading to challenges with their use across the broad RA patient population (47, 48). For example, one recent analysis of 1892 consecutive patients seen by 7 rheumatologists from Finland and the United States over 25 years demonstrated that ESR and CRP were normal at presentation in 35% to 45% of patients with RA (49).

Monitoring structural damage, meanwhile, relies on various imaging techniques, each with its own advantages and limitations (50). Radiography (x-rays and CT scans) are the conventional means of assessing structural damage and are readily available. However, relatively low sensitivity limits the monitoring frequency to once or twice a year at most, so that changes in skeletal progression due to disease alteration or treatment cannot be rapidly assessed. Furthermore, regular assessment using radiography in a chronic disease such as RA implies periodic, life-long exposure to radiation. Finally, as lagging indicators of damage, these techniques report established damage after the fact – often too late for prevention or mitigation. Magnetic resonance imaging and ultrasonography, while also lagging indicators of joint damage, are more sensitive than radiography and therefore exhibit less delay between the onset of erosive activity and evidence of damage on imaging. These modalities, however, require highly specialised expertise and expensive equipment (in the case of MRI), resulting in limited availability and significant inter- and intra-assessor variability.

Thus, better tools for measuring both disease activity and structural damage could complement existing approaches and facilitate more widespread use of "tight control" and "treat to target" strategies for chronic disease management in RA, with the ultimate goal of improving long-term patient outcomes. One promising approach that has garnered much attention recently is the use of biomarkers for evaluating RA disease activity and structural damage progression.

Novel biomarker assays in other therapeutic areas

Biomarker assays are fundamental to the diagnosis, monitoring, and treatment of many diseases, including various cancers, coronary artery disease, diabetes, and HIV (51-57). In addition to individual markers, in diseases with multi-factorial etiologies such as cancer and autoimmune diseases, multi-biomarker assays can simultaneously assess multiple disease pathways that may contribute to an individual patient's disease. For example, a 21-gene RT-PCR assay (Oncotype DX®; Genomic Health, Inc., Redwood City, California) has been validated to predict recurrence risk and chemotherapy benefit in early-stage breast cancer (58).

Similarly, in the field of immune response, a 20-gene RT-PCR assay (AlloMap®; XDx Inc., Brisbane, California), which was validated to identify heart transplant recipients who are at low probability of rejecting their trans-planted heart, has been incorporated in clinical practice and the International Society of Heart and Lung Transplant guidelines to minimise the need for (and risks associated with) periodic, invasive cardiac biopsies (59, 60).

Lastly, a novel serum-based, seven-biomarker test (PreDx Diabetes Risk Score model; Tethys Bioscience; Emeryville, California) has been validated to predict the risk of developing type 2 diabetes within a 5-year period (61). The PreDx model also outperformed additional measures such as haemoglobin A1c, fasting insulin, and a non-invasive clinical model including age, gender, BMI, waist circumference, and family history. The validation and adoption of these tests confirms the value of multi-biomarker approaches in complex, multi-faceted diseases.

Novel biomarkers in RA – individual biomarker assays

Rheumatoid arthritis is a complex, multi-factorial, and heterogeneous disease, with significant inter-patient variation in symptoms, natural history/disease course, and therapeutic response (62). Multiple cell types, tissues, and compartments are involved in RA pathophysiology, both within the affected joints and in the periphery (Fig. 1). The biological pathways primarily responsible for driving erosive activity and disease activity can vary from patient to patient and over time within a single patient (63). Studies have suggested different biological patterns in RA, from gene expression to synovial architecture, intrinsic (*e.g.* race, ethnicity, and gender) and extrinsic/environmental (*e.g.* diet and exercise) factors influence the contributions and interactions of different biological pathways – and ultimately the disease course – of RA in each individual (64-69).

For example, diffuse synovitis is seen more in patients with seronegative RA, while extra-articular spreading of RA with nodule formation is typically associated with granulomatous synovitis (70). Another study has showed that RA patients with synovial lymphocyte aggregates generally have a better response to tumour necrosis factor (TNF) inhibitor treatment (*e.g.* infliximab)
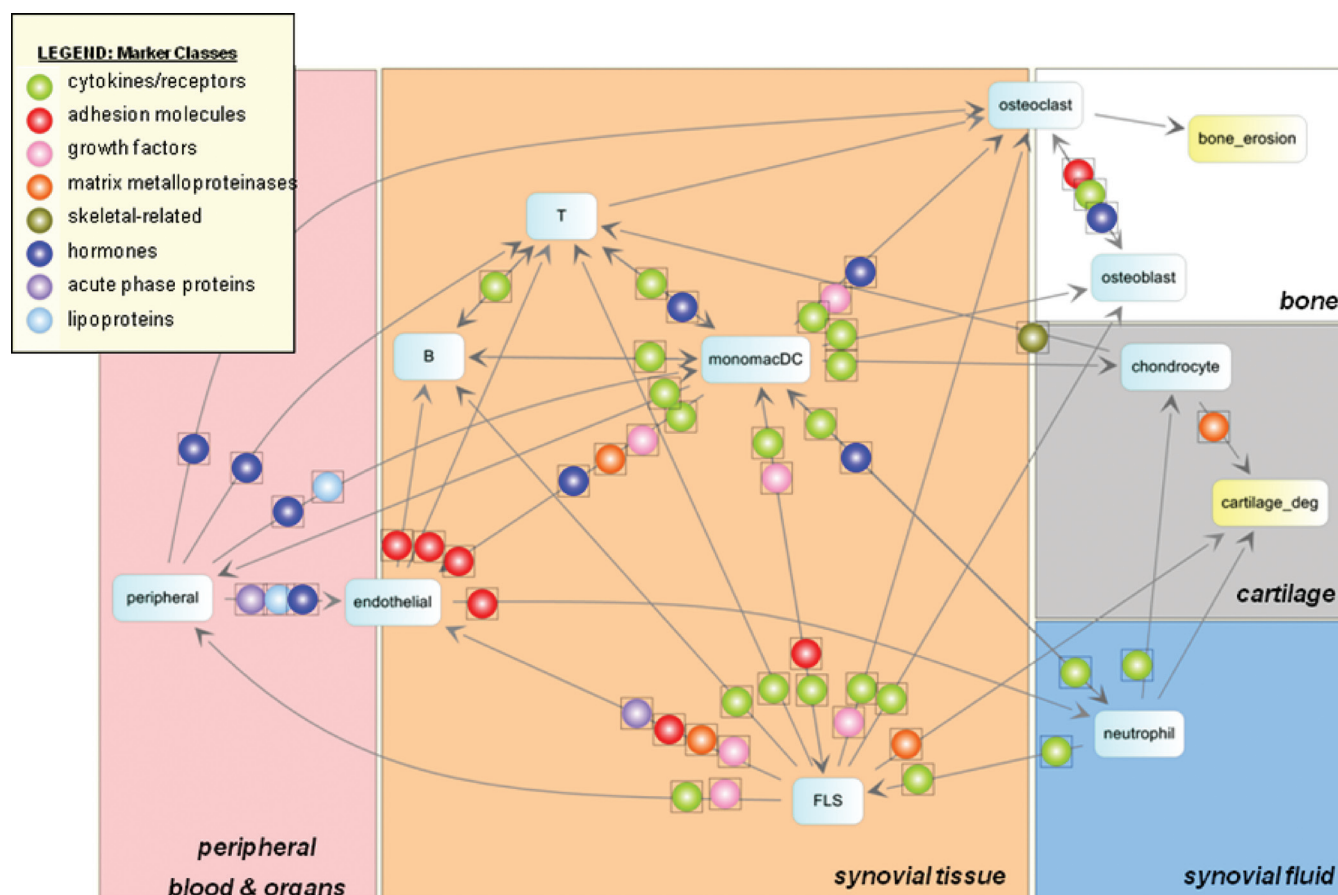
Fig. 1. Distinct classes of molecules driving different types of interactions in RA.
B, B cells; DC, dendritic; FLS, fibroblast-like synovial cells; T, T cells.

than those with only diffuse leukocyte infiltration (71). These studies and numerous others in the existing literature provide evidence of the heterogeneous nature of RA and suggest the existence of distinct pathogenic mechanisms that contribute to RA. Therefore, an understanding of the biological basis of disease activity and structural damage requires evaluation of the activity of multiple pathways.

Today, the only laboratory tests regularly used in the assessment of disease activity in RA are CRP and ESR (72-74), which are also utilised to calculate disease activity scores, such as the DAS28-ESR and the DAS28-CRP. However, as non-specific indicators of the peripheral response to inflammation rather than the processes driving RA pathophysiology, taken alone, these markers provide an incomplete picture of disease activity. Other biomarkers currently under investigation may capture additional components of the underlying biology

of synovial inflammation and hyperplasia, cartilage degradation, and bone erosion (Fig. 1). For instance, multiple cytokines clearly contribute to RA, possibly differently in different patients, as evidenced by the success of cytokine-inhibiting therapies, such as TNF inhibitors, and IL-6 receptor antagonists in anti-TNF inadequate responders (75). Similarly, the vascular contribution to RA involves adhesion molecules, chemokines, and angiogenic factors (*e.g.* VEGF), while skeletal degradation appears to be driven by cytokines and matrix metalloproteinases (MMPs). The complexity of these biological interactions and their heterogeneity among patients suggest that multiple analytes may more accurately characterise the disease state (76, 77).

Indeed, numerous circulating biomarkers have been associated with disease activity in RA in individual studies, though none have yet gone through rigorous validation or been proven useful

for clinical care (Table II). Serum amyloid A (SAA), for example, has been correlated with CRP (78). In a study of 185 patients with RA, SAA showed a greater incremental increase than CRP and was elevated in 40% of patients with normal CRP concentrations. These findings suggest that SAA may be a more sensitive marker of inflammation – and disease activity – than CRP. YKL-40, or human cartilage glycoprotein 39, is another often-studied marker, which is secreted primarily by chondrocytes and differentiated macrophages, and may promote chondrocyte and fibroblast proliferation and antagonise cartilage destruction (79-82). In a 1-year longitudinal study of 156 patients with RA, YKL-40 was increased in 54% of the patients with clinically active disease (83). Patients who changed from inactive to active disease after 12 months experienced an increase in serum YKL-40, while patients who changed from clinically active to inactive disease had

Table II. Selected potential biomarkers in RA.

Genetic markers	Human leukocyte antigen (HLA)-D4
	HLA-DRB-1
	Non-HLA markers
Autoantibodies	Rheumatoid factor (RF)
	Anti-cyclic citrullinated peptide (CCP)
	Mean corpuscular volume (MCV)
Markers of inflammation	Erythrocyte sedimentation rate (ESR)
	C-reactive protein (CRP)
	Interleukin (IL)-1
	IL-6
	IL-8
	Serum amyloid A (SAA)
	Vascular endothelia growth factor (VEGF)
	Tumour necrosis factor (TNF)-α
	YKL-40 (cartilage glycoprotein 39)
Markers of damage/destruction	Metalloproteinases (*e.g.* MMP-3), cartilage oligomeric matrix protein (COMP)
	Synovial markers: urinary glucosyl-galactosyl pyridinoline
	Cartilage markers: carboxy-terminal crosslinking telopeptide (CTC) of type 2 collagen; aggrecan, hyaluronic acid
	Bone markers: CTC of type 1 collagen; pyridinoline crosslinks; bone sialoprotein; receptor activator for nuclear factor $\varkappa\beta$ ligand

a 30% decrease in serum YKL-40. The study also found that YKL-40 decreased rapidly (24% after 7 days) during prednisolone therapy, and more slowly in patients treated with methotrexate only (15% after 60 days). This study indicated that YKL-40 is influenced by disease activity in RA and may provide information different from the conventional CRP and ESR markers. In another prospective study, VEGF concentrations were measured in 38 healthy subjects and 40 patients with RA (84). Higher VEGF levels were associated with high disease activity in RA, independent of age and gender.

Several biomarkers have also been identified as potential indicators of structural damage in RA. For example, MMP-3 was assessed in a longitudinal study that followed 109 patients with recent-onset RA for 2 years (85). Regression analysis showed that serum MMP-3 levels at disease onset were independently associated with the progression of joint damage (p=0.001). The MMP-3 levels were also shown to be significantly higher in the group with severe joint damage. Similarly, rheumatoid factor (RF), which has long been used for the diagnosis of RA, was reported to be an independent predictor of joint deterioration over 5 years (86). Other putative markers of structural damage include receptor activator

for nuclear factor κB ligand (RANKL), cartilage oligomeric matrix protein, urinary C-terminal cross linking telopeptide of type 1 collagen (CTX-I), CTX-II, and anticitrullinated protein antibodies (87-91).

Novel biomarkers in RA – multi-biomarker tests

Given the biological heterogeneity of RA, multi-biomarker tests have the potential to improve disease assessment. Rioja *et al.* (92) assessed 44 RA patients with proteomic arrays to determine a set of biomarkers which could distinguish between active and quiescent disease. Ninety-two analytes were assessed in plasma, resulting in 18 proteins which were statistically significantly up-regulated in active vs. quiescent RA (FDR ≤0.005). In multivariate modelling, a combination of 6 biomarkers showed promise in discriminating disease activity (IL-6, chemokine [C-X-C motif] ligand 13 [CXCL13], chemokine [C-C motif] ligand 23 [CCL23], transforming growth factor alpha, TNF receptor superfamily member 9, and macrophage colony-stimulating factor) with 91% predictive value for active *vs.* quiescent disease, moderately higher than that of IL-6 alone. Separately, Young-Min and *et al*. (93) assessed the contribution of novel biomarkers to predict radiographic progression in 118 subjects

with early RA. Biomarkers including MMPs, urinary collagen markers, and COMP were assessed, along with traditional variables such as physician global assessment, swollen and tender joint counts, baseline Larsen Score and DAS28, by univariate analysis and by multivariate logistic regression. Both clinical variables and biomarkers were predictive at baseline and in an AUC-based longitudinal model in univariate analysis. A multivariate model containing MMP-3 and urinary CTX-II yielded statistically significant results (p<0.001) with an AUC of the ROC of 0.76; a model including these 2 biomarkers and swollen joint count had even greater performance, with AUC of the ROC=0.81 (using either a 44- or 28-swollen joint count). Studies to further validate these and other proposed biomarker combinations and to confirm benefit beyond existing measures, must be conducted in larger, independent cohorts of RA patients. More recently, a new test for RA disease activity, which includes 12 biomarkers (Vectra™ DA; Crescendo Bioscience; South San Francisco, California), has been developed and validated; this work also followed a stepwise process of biomarker discovery (94), algorithm development and finalisation (95), and finally validation in an independent cohort (96). These types of multi-biomarker assays have the potential to provide rheumatologists with valuable, quantitative information on the individual disease biology of their patients.

As biomarker research evolves, it is important for clinicians to have a framework for assessing the clinical validity and clinical utility of novel assays. The Outcome Measures in Rheumatology Soluble Biomarkers Working Group of OMERACT has published draft guidelines on criteria for a single soluble biomarker of structural damage and includes measures of truth, discrimination, and feasibility (97, 98). Other groups have written on the optimal development and validation of multi-biomarker-based tests for diseases in general, including statistical methods and requirements for studies that now routinely explore correlations of tens, if not thousands, of biomarkers in a single

experiment (99, 100). In general, the authors of these publications agree that the development of biomarker assays requires the use of multiple, independent, sizable cohorts to both develop and validate a novel test, in order to reduce false discoveries and ensure that these new tests are reproducible across the relevant population. There is also general agreement that both analytical validity (accuracy and reproducibility) and clinical validity must be ensured.

Conclusion

Regular assessment of disease activity and structural damage is recommended by rheumatologists and guideline committees around the world. Tight control strategies employing regular measurement with resulting treatment changes designed to reach a specific disease activity goal have shown improvements in patient outcomes. Objective, precise, and easy-to-use measures which can standardise assessment of disease activity and skeletal damage progression in patients with RA have the potential to further improve patient outcomes. Extensive research is ongoing to better understand biomarkers that drive biological processes underlying disease activity and structural damage. Until now, individual biomarkers have not been shown to adequately capture the complexity and heterogeneity of RA and hence are not heavily relied upon in clinical practice. However, coordinated, quantitative measurement of multiple biomarkers associated with different aspects of the disease may encompass this heterogeneity. As shown in other disease states, such technology has the potential to complement existing measures and enable more consistent and comprehensive evaluation of an individual patient's disease. Such tools can foster the systematic implementation of tight control strategies, culminating improved long-term outcomes and quality of life for patients suffering from RA.

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References

1. ALAMANOS Y, VOULGARI, P, DROSOS A: Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. *Semin Arthritis Rheum* 2006; 36: 182-8.
2. KVIEN TK: Epidemiology and burden of illness of rheumatoid arthritis. *Pharmacoeconomics* 2004; 22 (Suppl. 2): 1-12.
3. FEELY MG, ERICKSON A, O'DELL JR: Therapeutic options for rheumatoid arthritis. *Expert Opin Pharmacother* 2009; 10: 2095-106.
4. ISAACS JD: Therapeutic agents for patients with rheumatoid arthritis and an inadequate response to tumour necrosis factor-alpha antagonists. *Expert Opin Biol Ther* 2009; 9: 1463-75.
5. MAINI R, ST CLAIR EW, BREEDVELD F *et al.*: Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999; 354: 1932-9.
6. CROWSON CS, MYASOEDOVA E, MATTESON EL, KREMERS HM, THERNEAU TM, GABRIEL SE: Has survival increased in patients diagnosed with rheumatoid arthritis? Programs and abstracts of the 2009 ACR/ARHP Annual Meeting; October 21, 2009; Philadelphia, PA. Abstract 1172.
7. GABRIEL SE, CROWSON CS, O'FALLON WM: Mortality in rheumatoid arthritis: have we made an impact in 4 decades? *J Rheumatol* 1999; 26: 2529-33.
8. KATCHAMART W, BOMBARDIER C: Systematic monitoring of disease activity using an outcome measure improves outcomes in rheumatoid arthritis. *J Rheumatol* 2010; 37: 1411-5.
9. BAKKER MF, JACOBS JW, VERSTAPPEN SM, BIJLSMA JW: Tight control in the treatment of rheumatoid arthritis: efficacy and feasibility. *Ann Rheum Dis* 2007; 66 (Suppl. III): iii56-iii60.
10. SMOLEN JS, ALETAHA D, BIJLSMA JW *et al.*: T2T Expert Committee: Treating rheumatoid arthritis to target: recommendations of an international task force. *Ann Rheum Dis* 2010; 69: 631-7.
11. PETERSON KA, SMITH CK: The DCCT findings and standards of care for diabetes. *Am Fam Physician* 1995; 52: 1092-8.
12. GENUTH S: Insights from the diabetes control and complications trial/epidemiology of diabetes interventions and complications study on the use of intensive glycemic treatment to reduce the risk of complications of type 1 diabetes. *Endocr Pract* 2006; 12 (Suppl. 1): 34-41.
13. MOTTONEN T, HANNONEN P, LEIRISALO-REPO M *et al.*: Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomized trial. FIN-RACo trial group. *Lancet* 1999; 353: 1568-73.
14. GRIGOR C, CAPELL H, STIRLING A *et al.*: Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *Lancet* 2004; 364: 263-9.
15. GOEKOOP-RUITERMAN YP, DE VRIES-BOUWSTRA JK, ALLAART CF *et al.*: Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study). *Arthritis Rheum* 2005; 52: 3381-90.
16. VERSTAPPEN SM, JACOBS JW, VAN DER VEEN MJ *et al.*: Intensive treatment with methotrexate in early rheumatoid arthritis: aiming for remission. Computer Assisted Management in Early Rheumatoid Arthritis (CAMERA, an open-label strategy trial). *Ann Rheum Dis* 2007; 66: 1443-9.
17. SMOLEN JS, LANDEWÉ R, BREEDVELD FC *et al.*: EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann Rheum Dis* 2010; 69: 964-75.
18. ALETAHA D: EULAR/ACR collaborative recommendations. *Arthritis Rheum* 2008; 59: 1371-7.
19. JALAVA S, REUNANEN K: Healing of erosions in rheumatoid arthritis. *Scand J Rheumatol* 1982; 11: 97-100.
20. REDLICH K, GÖRTZ B, HAYER S *et al.*: Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. *Am J Pathol* 2004; 164: 543-55.
21. HETLAND ML, EJBJERG B, HØRSLEV-PETERSEN K *et al.*: MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2 year randomized controlled trial (CIMESTRA). *Ann Rheum Dis* 2009; 68: 384-90.
22. KIRWAN JR: The relationship between synovitis and erosions in rheumatoid arthritis. *Br J Rheumatol* 1997; 36: 225-8.
23. KIRWAN JR: The synovium in rheumatoid arthritis: evidence for (at least) two pathologies. *Arthritis Rheum* 2004; 50:1-4.
24. COHEN G, GOSSEC L, DOUGADOS M *et al.*: Radiological damage in patients with rheumatoid arthritis on sustained remission. *Ann Rheum Dis* 2007; 66: 358-63.
25. SVENSSON B, SCHAUFELBERGER C, TELEMAN A, THEANDER J: Remission and response to early treatment of RA assessed by the Disease Activity Score. BARFOT study group. Better Anti-rheumatic Pharmacotherapy. *Rheumatology* 2000; 39: 1031-6.
26. MOTTONEN T, HANNONEN P, KORPELA M *et al.*: Delay to institution of therapy and induction of remission using single-drug or combination-disease-modifying antirheumatoid drug therapy in early rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 894-8.
27. SMOLEN JS, BREEDVELD FC, SCHIFF MH *et al.*: A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology* (Oxford) 2003; 42: 244-57.
28. PREVOO ML, VAN 'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LB, VAN RIEL PL: Modified disease activity scores that include twenty-eight-joint counts.

Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-8.

29. KLINKHOFF AV, BELLAMY N, BOMBARDIER C *et al.*: An experiment in reducing interobserver variability of the examination for joint tenderness. *J Rheumatol* 1988; 15: 492-4.

30. UHLIG T, KVIEN TK, PINCUS T: Test-retest reliability of disease activity core set measures and indices in rheumatoid arthritis. *Ann Rheum Dis* 2008; 68: 972-5.

31. HART LE, TUGWELL P, BUCHANAN WW, NORMAN GR, GRACE EM, SOUTHWELL D: Grading of tenderness as a source of interrater error in the Ritchie articular index. *J Rheumatol* 1985; 12: 716-7.

32. HERNÁNDEZ-CRUZ B, CARDIEL MH: Intraobserver reliability of commonly used outcome measures in rheumatoid arthritis. *Clin Exp Rheumatol* 1998; 16: 459-62.

33. THOMPSON PW, HART LE, GOLDSMITH CH, SPECTOR TD, BELL MJ, RAMSDEN MF: Comparison of four articular indices for use in clinical trials in rheumatoid arthritis: patient, order and observer variation. *J Rheumatol* 1991; 18: 661-5.

34. MARHADOUR T, JOUSSE-JOULIN S, CHALÈS G *et al.*: Reproducibility of joint swelling assessments in long-lasting rheumatoid arthritis: influence on Disease Activity Score-28 values (SEA-Repro study part I). *J Rheumatol* 2010; 37: 932-7.

35. PINCUS T: Advantages and limitations of quantitative measures to assess rheumatoid arthritis: joint counts, radiographs, laboratory tests, and patient questionnaires. *Bull NYU Hosp Jt Dis* 2006; 64: 32-9.

36. PINCUS T, YAZICI Y, SOKKA T: Quantitative measures of rheumatic diseases for clinical research versus standard clinical care: differences, advantages and limitations. *Best Pract Res Clin Rheumatol* 2007; 21: 601-28.

37. ALETAHA D, SMOLEN J: The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI): a review of their usefulness and validity in rheumatoid arthritis. *Clin Exp Rheumatol* 2005; 23 (Suppl. 39): S100-S108.

38. FELSON DT, ANDERSON JJ, BOERS M *et al.*: The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. *Arthritis Rheum* 1993; 36: 729-40.

39. VAN GESTEL AM, HAAGSMA CJ, VAN RIEL PL: Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. *Arthritis Rheum* 1998; 41: 1845-50.

40. PREVOO ML, VAN GESTEL AM, VAN 'T HOF MA, VAN RIJSWIJK MH, VAN DE PUTTE LB, VAN RIEL PL: Remission in a prospective study of patients with rheumatoid arthritis. American Rheumatism Association preliminary remission criteria in relation to the disease activity score. *Br J Rheumatol* 1996; 35: 1101-5.

41. VANDER CRUYSSEN B, VAN LOOY S, WYNS B *et al.*: DAS28 best reflects the physician's clinical judgment of response to infliximab therapy in rheumatoid arthritis patients: validation of the DAS28 score in patients under infliximab treatment. *Arthritis Res Ther* 2005; 7: R1063-71.

42. SMOLEN JS, HAN C, BALA M *et al.*: 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* 2005; 52: 1020-30.

43. WEINBLATT ME, KEYSTONE EC, FURST DE, KAVANAUGH AF, CHARTASH EK, SEGURADO OG: Long term efficacy and safety of adalimumab plus methotrexate in patients with rheumatoid arthritis: ARMADA 4 year extended study. *Ann Rheum Dis* 2006; 65: 753-9.

44. VAN DER HEIJDE DM, VAN 'T HOF M, VAN RIEL PL, VAN DE PUTTE LB: Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* 1993; 20: 579-81.

45. DOUGADOS M, ALETAHA D, VAN RIEL P: Disease activity measures for rheumatoid arthritis. *Clin Exp Rheumatol* 2007; 25 (5 Suppl. 46): S22-9.

46. PINCUS T, MACLEAN R, YAZICI Y, HARRINGTON JT: Quantitative measurement of patient status in the regular care of patients with rheumatic diseases over 25 years as a continuous quality improvement activity, rather than traditional research. *Clin Exp Rheumatol* 2007; 25 (Suppl. 47): 69-81.

47. PINCUS T, SOKKA T: Complexities in the quantitative assessment of patients with rheumatic diseases in clinical trials and clinical care. *Clin Exp Rheumatol* 2005; 23 (Suppl. 39): S1-S9.

48. PINCUS T, SEGURADO O: Most visits of most patients with rheumatoid arthritis to most rheumatologists do not include a formal quantitative joint count. *Ann Rheum Dis* 2006; 65: 820-2.

49. SOKKA T, PINCUS T: Erythrocyte sedimentation rate, C-reactive protein, or rheumatoid factor are normal at presentation in 35% to 45% of patients with rheumatoid arthritis seen between 1980 and 2004: analyses from Finland and the United States. *J Rheumatol* 2009; 36: 1387-90.

50. YAZICI Y, SOKKA T, PINCUS T: Radiographic measures to assess patients with rheumatoid arthritis: advantages and limitations. *Rheum Dis Clin North Am* 2009; 35: 723-9.

51. ROSS JS, HATZIS C, SYMMANS WF, PUSZTAI L, HORTOBÁGYI GN: Commercialized multigene predictors of clinical outcome for breast cancer. *Oncologist* 2008; 13: 477-93.

52. CHEN CH, YU DT, CHOU CT: Biomarkers in spondyloarthropathies. *Adv Exp Med Biol* 2009; 649: 122-32.

53. LITTLE RR, SACKS DB: HbA1c: how do we measure it and what does it mean? *Curr Opin Endocrinol Diabetes Obes* 2009; 16: 113-8.

54. GOLLEDGE AL, WALKER P, NORMAN PE, GOLLEDGE J: A systematic review of studies examining inflammation associated cytokines in human abdominal aortic aneurysm samples. *Dis Markers* 2009; 26: 181-8.

55. WOELK CH, BELIAKOVA-BETHELL N, GOICOECHEA M *et al.*: Gene expression before HAART initiation predicts HIV-infected individuals at risk of poor CD4+ T-cell recovery. *AIDS* 2010; 24: 217-22.

56. LEITZEL K, LIPTON A, KOESTLER W *et al.*: Use of total HER2 and HER2 homodimer levels to predict response to trastuzumab. *J Clin Oncol* 2008; 26 (20 Suppl.): Abstract 1002.

57. MERX K, MÜLLER MC, KREIL S *et al.*: Early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in chronic phase CML patients treated with imatinib and failure of interferon alpha. *Leukemia* 2002; 16: 1579-83.

58. PAIK S, SHAK S, TANG G *et al.*: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; 351: 2817-26.

59. EISEN HJ, DEN MC, KLINGER TM *et al.*: Longitudinal monitoring of cardiac allograft recipients using peripheral blood gene expression profiling: a retrospective observational analysis of molecular testing in 19 case studies. *J Heart Lung Transplant* 2005; 24 (2 Suppl.): S162.

60. COSTANZO MC, COSTANZO MR, DIPCHAND A: The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant* 2010; 29: 914-56.

61. URDEA M, KOLBERG J, WILBER J *et al.*: Validation of a multimarker model for assessing risk of type 2 diabetes from a five-year prospective study of 6784 Danish people (Inter99). *J Diabetes Sci Technol* 2009; 3: 748-55.

62. CHOY E, PANAYI GS: Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001; 344: 907-16.

63. MARKATSELI TE, PAPAGORAS C, DROSOS AA: Prognostic factors for erosive rheumatoid arthritis. *Clin Exp Rheumatol* 2010; 28: 114-23.

64. WEYAND CM, XIE C, GORONZY JJ: Homozygosity for the HLA-DRB1 allele selects for extraarticular manifestations in rheumatoid arthritis. *J Clin Invest* 1992; 89: 2033-9.

65. ALAMANOS Y, DROSOS AA: Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005; 4: 130-6.

66. MIKULS TR, HUGHES LB, WESTFALL AO *et al.*: Cigarette smoking, disease severity and autoantibody expression in African Americans with recent-onset rheumatoid arthritis. *Ann Rheum Dis* 2008; 67: 1529-34.

67. PLENGE RM, COTSAPAS C, DAVIES L *et al.*: Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat Genet* 2007; 39: 1477-82.

68. RAYCHAUDHURI S, REMMERS EF, LEE AT *et al.*: Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008; 40: 1216-23.

69. URSUM J, VAN DER HORST IE, BOERS M, DIJKMANS BA, VAN SCHAARDENBURG D: Gender differences in the presentation of complaints may affect the Disease Activity Score (DAS28). Programs and abstracts of the 2008 ACR/ARHP Annual Meeting;

October 29, 2008; San Francisco, CA. Abstract 1602.

70. KLIMIUK PA, GORONZY JJ, BJÖRNSSON J, BECKENBAUGH RD, WEYAND CM: Tissue cytokine patterns distinguish variants of rheumatoid synovitis. *Am J Pathol* 1997; 151: 1311-9.

71. KLAASEN R, THURLINGS RM, WIJBRANDTS CA *et al.*: The relationship between synovial lymphocyte aggregates and the clinical response to infliximab in rheumatoid arthritis: a prospective study. *Arthritis Rheum* 2009; 60: 3217-24.

72. KEELING SO, LANDEWÉ R, VAN DER HEIJDE D *et al.*: Testing of the preliminary OMERACT validation criteria for a biomarker to be regarded as reflecting structural damage endpoints in rheumatoid arthritis clinical trials: the example of C-reactive protein. *J Rheumatol* 2007; 34: 623-33.

73. WELLS G, BECKER JC, TENG J *et al.*: Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis* 2009; 68: 954-60.

74. DE RYCKE L, PEENE I, HOFFMAN IE *et al.*: Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. *Ann Rheum Dis* 2004; 63: 1587-93.

75. BRENNAN FM, MCINNES IB: Evidence that cytokines play a role in rheumatoid arthritis. *J Clin Invest* 2008; 118: 3537-45.

76. DHAOUADI T, SFAR I, ABELMOULA L *et al.*: Role of immune system, apoptosis and angiogenesis in pathogenesis of rheumatoid arthritis and joint destruction, a systematic review. *Tunis Med* 2007; 85: 991-8.

77. SMOLEN JS, ALETAHA D, GRISAR J, RED-LICH K, STEINER G, WAGNER O: The need for prognosticators in rheumatoid arthritis. Biological and clinical markers: where are we now? *Arthritis Res Ther* 2008; 10: 208.

78. CHAMBERS RE, MACFARLANE DG, WHICH-ER JT, DIEPPE PA: Serum amyloid-A protein concentration in rheumatoid arthritis and its role in monitoring disease activity. *Ann Rheum Dis* 1983; 42: 665-7.

79. KIRKPATRICK RB, EMERY JG, CONNOR JR, DODDS R, LYSKO PG, ROSENBERG M: Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. *Exp Cell Res* 1997; 237: 46-54.

80. DE CEUNINCK F, GAUFILLIER S, BONNAUD A, SABATINI M, LESUR C, PASTOUREAU P: YKL-40 (cartilage gp-39) induces proliferative events in cultured chondrocytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. *Biochem Biophys Res Commun* 2001; 285: 926-31.

81. LING H, RECKLIES AD: The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. *Biochem J* 2004; 380: 651-9.

82. KOTZIN BL, FALTA MT, CRAWFORD F *et al.*: Use of soluble peptide-DR4 tetramers to detect synovial T cells specific for cartilage antigens in patients with rheumatoid arthritis. *Proc Natl Acad Sci U S A* 2000; 97: 291-6.

83. JOHANSEN JS, STOLTENBERG M, HANSEN M *et al.*: Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. *Rheumatology* (Oxford) 1999; 38: 618-26.

84. OZGONENEL L, CETIN E, TUTUN S, TONBAKLAR P, ARAL H, GUVENEN G: The relation of serum vascular endothelial growth factor level with disease duration and activity in patients with rheumatoid arthritis, *Clin Rheumatol* 2010; 29: 473-7.

85. TCHETVERIKOV I, LARD L, DEGROOT J *et al.*: Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. *Ann Rheum Dis* 2003; 62: 1094-9.

86. BUKHARI M, LUNT M, HARRISON BJ, SCOTT DG, SYMMONS DP, SILMAN AJ: Rheumatoid factor is the major predictor of increasing severity of radiographic erosions in rheumatoid arthritis. *Arthritis Rheum* 2002; 46: 906-12.

87. GEUSENS PP, LANDEWÉ RB, GARNERO P *et al.*: The ratio of circulating osteoportegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006; 54: 1772-7.

88. VAN DER HELM-VAN MIL AH, VERPOORT KN, BREEDVELD FC, TOES RE, HUIZINGA TW: Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005; 7: R949-58.

89. LANFANT K, MICHOT C, DAVEAU R *et al.*: An algorithm including the intrasynovial expression of CD20 and serum biomarkers (rheumatoid factors, anti-CCP2 and RANK-ligand) able to predict the progression of bone erosions in very early arthritis. Programs and abstracts of the 2008 ACR/ARHP Annual Meeting; October 29, 2008; San Francisco, CA. Abstract 1605.

90. NAM J, VILLENUEVE E, EMERY P: The role of biomarkers in the management of patients with rheumatoid arthritis. *Curr Rheumatol Rep* 2009; 11: 371-7.

91. SYVERSEN SW, LANDEWÉ R, VAN DER HEIJDE D *et al.*: Testing of the OMERACT 8 draft validation criteria for a soluble biomarker reflecting structural damage in rheumatoid arthritis: a systematic literature search on 5 candidate biomarkers. *J Rheumatol* 2009; 36: 1769-84.

92. RIOJA I, HUGHES FJ, SHARP CH *et al.*: Potential novel biomarkers of disease activity in rheumatoid arthritis patients: CXCL12, CCL23, transformation growth factor alpha, tumor necrosis factor receptor superfamily member 9, and macrophage colony-stimulating factor. *Arthritis Rheum* 2008; 58: 2257-67.

93. YOUNG-MIN, CAWSTON T, MARSHALL N *et al.*: Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. *Arthritis Rheum* 2007; 56: 3236-47.

94. SHEN Y, KNOWLTON N, TURNER M *et al.*: Stepwise development of a multi-protein biomarker index of RA disease activity. Programs and abstracts of the 2009 ACR/ARHP Annual Meeting; October 21, 2009; Philadelphia, PA. Abstract 345.

95. CAVET G, CENTOLA M, SHEN Y *et al.*: Development of a multi-biomarker test for rheumatoid arthritis (RA) disease activity. Presented at the 2010 EULAR Congress; *Ann Rheum Dis* 2010; 69 (Suppl. 3): 148.

96. CURTIS JR, HANEY D, VAN DER HELM AHM *et al.*: Validation of a multi-biomarker test for rheumatoid arthritis (RA) disease activity (Vectra DA) in a multi-cohort study. Programs and abstracts of the 2010 ACR/ARHP Annual Meeting; November 6-11, 2010; Atlanta, GA. Abstract 2566. [Manuscript in development]

97. MAKSYMOWYCH WP, FITZGERALD O, WELLS GA *et al.*: Proposal for levels of evidence schema for validation of a soluble biomarker reflecting damage endpoints in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis, and recommendations for study design. *J Rheumatol* 2009; 36: 1792-99.

98. MAKSYMOWYCH WP, LANDEWÉ R, TAK PP *et al.*: Reappraisal of OMERACT 8 draft validation criteria for a soluble biomarker reflecting structural damage endpoints in rheumatoid arthritis, psoriatic arthritis, and spondyloarthritis: the OMERACT 9 v2 criteria. *J Rheumatol* 2009; 36: 1785-91.

99. SIMON R: Development and validation of biomarker classifiers for treatment selection. *J Stat Plan Inference* 2008; 138: 308-20.

100. SMOLEN JS, ALETAHA D: Developments in the clinical understanding of rheumatoid arthritis. *Arthritis Res Ther* 2009; 11: 204.