OPTIMISING REMISSION AND TREATMENT OUTCOMES IN RHEUMATOID ARTHRITIS

by

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SUMMARY

Rheumatoid arthritis (RA) is a chronic, debilitating autoimmune disorder affecting 1-2% of the population. Despite the advent of newer therapies, in combination with a treat-to-target approach, sustained remission remains elusive for a significant proportion of patients.

This thesis explores measures to improve outcomes in early RA, a time when therapies are most likely to achieve sustained remission. The first section evaluates commonly used disease activity (DA) measures that omit assessment of the foot and ankle, a frequent site of joint involvement. Our initial cross-sectional study, demonstrates persistent foot synovitis in >20% of patients meeting standard remission criteria. Our subsequent longitudinal study found that when DA measures using 28 joint counts are used to define remission, a substantial proportion of patients have ongoing foot synovitis and this in turn predicts relapse and radiographic progression. We also found an independent association between foot synovitis and the short form-36 physical functioning subscale, underscoring the importance of foot synovitis in activities of daily living.

We next addressed whether contemporary treat-to-target combination DMARD therapy in early RA translates into personal and societal benefits in terms of preserved work outcomes. Our findings revealed that good EULAR responders were more likely to be working at 10 years compared to those with moderate/no EULAR response. This difference was present from 2 years following diagnosis and became more pronounced over the next 8 years.

Dissociation between radiographic progression and apparent remission can lead to unexpected treatment failures, particularly in the setting of biologic DMARD (bDMARD) therapy. We wished to identify these patients early in their disease. We explored whether inclusion of bone biomarkers could improve assessment of treatment response. We found a significant reduction of RANKL following treatment, a slight increase in osteoprotegerin (OPG), and no significant changes in the other bone biomarkers assessed.

The RA synovium reflects the underlying cytokine milieu in each individual and in the final section of this thesis we detail our ongoing research exploring the clinical utility of arthroscopic biopsy in early RA. We initially discuss a proof-of-concept study followed by the currently ongoing ARBITRATE (Arthroscopic Synovial Biopsy Directed Targeted Therapy vs. Conventional Therapy in RA) study, an open label randomised parallel design

treatment trial designed to address whether targeted therapy can improve disease outcome. We conclude with a critical review of the findings from this thesis and future research directions.

DECLARATION

I certify that this thesis does not contain, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person, except where due reference is made in the text.

……..

Dr Mihir Dilip Wechalekar

I believe that this thesis is properly presented, conforms to the specifications of thesis presentation in the University and is prima facie worthy of examination.

……..

Professor Malcolm D Smith

Dedicated to

my parents, for inculcating in me my love for academics and inspiring me to strive for excellence

my wife Harsha, for her love and support

and my daughters Gauri and Isha, for making everything worthwhile

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PUBLICATIONS AND CONFERENCE PRESENTATIONS ARISING FROM THE WORK CONTAINED IN THIS THESIS

- **Wechalekar MD**, Lester S, Hill CL, Lee A, Rischmueller M, Smith MD, Walker JG, Proudman SM. Foot synovitis in patients with rheumatoid arthritis in apparent remission is associated with unstable remission status, radiographic progression and worse long-term functional outcomes. Submitted to Arthritis Care & Research (2015).
- **Wechalekar MD**, Lester S, Hill CL, Rischmueller M, Proudman SM. Foot synovitis in patients with rheumatoid arthritis in apparent remission is associated with unstable remission status, radiographic progression and worse long-term functional outcomes. Oral presentation at the American College of Rheumatology (ACR), Annual Meeting, San Francisco, USA (2015).
- **Wechalekar MD**, Quinn S, Lester S, Metcalf RG, Shanahan E, Walker JG, Smith MD, Hill CL, Shanahan EM, Proudman SM. A treat-to-target strategy preserves work capacity in early rheumatoid arthritis. Poster presentation at the American College of Rheumatology (ACR), Annual Meeting, Boston, USA (2014).
- **Wechalekar MD**, Quinn S, Lester S, Metcalf RG, Shanahan E, Walker JG, Smith MD, Hill CL, Shanahan EM, Proudman SM. Work disability in an inception cohort of early rheumatoid arthritis receiving treat-to-target therapy. Poster presentation at the Australian Rheumatology Association Annual Meeting, Hobart, Australia (2014).
- **Wechalekar MD**, Quinn S, Lester S, Metcalf RG, Shanahan E, Walker JG, Smith MD, Hill CL, Shanahan EM, Proudman SM. A treat-to-target strategy preserves work capacity in a rheumatoid arthritis inception cohort. Manuscript under preparation for submission to the Journal of Rheumatology.
- **Wechalekar MD**, Lester S, Proudman SM, Cleland LG, Whittle SL, Rischmueller M, Hill CL. Active foot synovitis: Criteria for remission and disease activity underestimate foot involvement in rheumatoid arthritis. Arthritis Rheum 2012;64(5):1316-22.

CHAPTER 1. INTRODUCTION

BACKGROUND AND PATHOGENESIS OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder affecting approximately 1-2% of the population with profound individual, societal and socio-economic consequences.[1,2] Given the considerable heterogeneity[3,4] in clinical presentation, serology, genetics and disease course; the term RA can be considered an umbrella term embracing multiple disease subtypes.[4] Despite extensive research, its precise aetiology has defied elucidation; it is thought to be the result of interaction between genetic factors, sex hormones and the immune system, possibly as a result of a microbial agent triggering the immune-inflammatory cascade.[5]

Following an appropriate trigger in a genetically predisposed individual, T-cells are activated by antigen-presenting dendritic cells (DC) and subsequently play a preeminent role in the initiation of immunopathology.[5,6] T-cells incite autoantibody production by B-cells,[7] and these autoantibodies form immune complexes that accumulate in joints and activate complement. Further recruitment of effector cells enhances production of pro-inflammatory cytokines and chemokines and activates osteoclastogenesis, causing cartilage and bone damage.[8]

By the time the patient presents with symptoms, this cascade is probably well underway, as demonstrated by the presence of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) autoantibodies years before the onset of clinical disease.[9,10]

Evidence of Autoimmunity: Autoantibodies in RA

Evidence of autoimmunity, characterised by AA and auto-reactive T cells in blood and synovial fluid, is one of the hallmarks of RA. Rheumatoid factor (RF) was first identified in blood and synovial tissue in 1922, yet its precise role in the pathogenesis of RA remains unclear. [11] RFs have a moderate sensitivity (60-80%) and specificity (~50%) for RA and are part of the 1987 and 2010 American College of Rheumatology (ACR) RA classification criteria.[12,13] They also have prognostic importance.[14]

More recently, autoantibodies to citrullinated peptides, anti-citrullinated protein antibodies (ACPA), have been recognised, with important diagnostic and prognostic implications.[10] This

discovery had its origins in reports from the 1970s when antibodies directed against keratin were detected in rheumatoid serum, and the primary target antigen was the filament-aggregating protein filaggrin. These antibodies bind to citrulline-containing epitopes on filaggrin. Citrulline is derived from post-translational modification of arginine residues by a process called as citrullination. Citrullination itself is a physiological process, believed to be important for degradation of intracellular proteins during apoptosis, and happens in the presence of high calcium concentrations by an enzyme called PAD (peptidyl arginine deiminase).[15] ACPA assays (which have a sensitivity of 60-70%) recognise several citrullinated self-proteins including α-enolase, keratin, fibrinogen, collagen and vimentin and are found almost exclusively in RA. The high specificity of ACPA for RA indicates that an as yet unknown mechanism specific for RA must exist that leads to a breakdown of tolerance to these citrullinated antigens. ACPA are usually IgG1, with the next most prominent subclass being IgG4, and to a lesser extent IgG2 and IgG3.[16] Of further interest is the fact that ACPA develop preferentially in patients that harbor the shared-epitope (SE) alleles.[17]

Carbamylation (in which lysines, under the influence of cyanate, are converted to homocitrullines) is another process that engenders post-translational protein modification in the context of chronic inflammation, and anti-carbamylated antibodies have been found in patients with RA. [18] In the context of chronic inflammation and tissue debris, elastase or cathepsin G associated protein cleavage of IgG antibodies can lead to neoepitope exposure at the IgG hinge region and result in anti-hinge antibodies (AHAs). Indeed, IgG4-AHAs have been identified at all stages of RA disease, and can complex with IgG4-ACPAs to further trigger inflammation.[19]

Genetic Risk Factors

The most compelling evidence for a genetic component comes from studies on twins: monozygotic twins have a concordance rate of 12-15% for RA, compared with 1% for the general population. The immunogenetics of RA are incompletely understood, but the best known and probably most influential factor is the HLA class II haplotype of an individual.

Several HLA-DRB1 molecules (*0101, *0401, *0404) share a common amino acid sequence at position 70-74 in the third hypervariable region of the DRß1 chain. This sequence, consisting of glutamine-leucine-arginine-alanine-alanine (QKRAA), is situated in the antigen binding cleft of class II HLA and has been termed the shared epitope (SE). Initially, the SE

was thought to bind a putative athritogenic peptide [20] but recent studies have shown that the shared amino acids actually face away from the antigen binding cleft. Analyses using genome-wide single nucleotide polymorphism data and conditional haplotype analyses revealed that the MHC association to RA risk (in the DR-B1 region) is almost completely explained by amino acids at positions 11, 71 and 74.[21] It is now thought that the SE may influence selection of a predisposing T-cell repertoire, antigen presentation or alteration in peptide affinity and therefore promote autoreactive adaptive immune responses.[22] Very recent work has shown that HLA DR B1*04:01/04 preferentially binds RA associated citrulline, and may lead to increased presentation to citrullinated self-antigen specific CD4+ T cells, which correlated with RA disease activity.[23]

Several other risk alleles that influence immune regulation have been identified, particularly in ACPA positive disease; these include alleles involved in nuclear factor-κB (NF-κB)-dependant signalling (e.g. TRAF1-C5 and c-REL), and T cell stimulation, activation and differentiation (e.g. PTPN-22 and CTLA4).[22]

Female predominance and pregnancy are known risk factors for RA suggesting that sex hormones and reproductive factors influence RA development and severity. Women with a lower age of menarche have a lower risk for development of RA while pregnancy and multiparity increase risk.[24]

Environmental Risk Factors

Smoking is strongly associated with RA, even in patients without a family history of RA, and is a prominent example of gene-environment interaction in RA pathogenesis, with the risk increasing with heavy smoking and particularly with those having the shared epitope alleles.[25-28] Indeed, citrullinated proteins have been detected in the bronchoalveolar lavage fluid of smokers, but not non-smokers.[29] Smoking may contribute to qualitative and quantitative alteration in citrullination (through PADI4) of mucosal proteins, epitope spreading and post-translational modification.[22]

Infections have been the subject of considerable, albeit inconclusive research as candidates for pathogen-derived peptides inducing autoimmunity by molecular mimicry. The most convincing evidence is from the observed association of RA with periodontal disease driven by *Porphyromonas gingivalis*. *P gingivalis* is unique in its ability to citrullinate host peptides

and may provide a mechanism for generating antigens that may further the autoimmune cascade.[30]

Adverse life events are a well-recognised trigger of RA; the central nervous system influences immune homeostasis, there is evidence of a link between the hypothalamic-pituitary-adrenal axis and cytokine production and finally, several neurotransmitters are expressed in the synovium.[22]

Immune dysregulation in RA

There are several candidate triggers which could initiate the immune-inflammatory cascade in RA. Initial events probably involve Toll-like receptors (TLRs) which play a role in pathogen recognition, leucocyte recruitment and induction of co-stimulatory molecules on professional antigen-presenting cells (APCs) such as dendritic cells (DCs).[5] Further events include several inflammatory cascades (perpetuated by inflammatory immune and synovial lining cells, cytokines and chemokines) culminating finally in persistent synovial inflammation.[31]

TLRs in RA

TLRs are germline-encoded pattern recognition receptors (PRRs) expressed on APCs and DCs (among others) and are considered to be the frontline host defence against harmful triggers. They are probably involved in initiating and maintaining inflammation in RA.[5,32] TLRs recognize exo- and endogenous ligands leading to activation of inflammatory signalling pathways, especially NFκB and mitogen-activated protein kinase (MAPK). Multiple TLRs (TLR2, TLR3, TLR4, TLR7, TLR8) are expressed in inflamed synovial tissue and inflammatory mediators expressed in inflamed joints [IL (interleukin)-12, IL-18] may up-regulate their expression. Furthermore, TLR ligands can induce tumour necrosis factor (TNF), IL-1, IL-6, IL-8 and matrix metalloproteinase (MMP) production by synovial cells. While the list of putative exo- and endogenous ligands recognized by TLRs on APCs or DCs and relevant to RA is growing, a definite candidate ligand awaits identification.[5,32]

Dendritic Cells in Rheumatoid Arthritis

Following uptake of immunogenic antigen or stimulation by TLR ligand, DCs undergo differentiation and maturation. They migrate to secondary lymphoid organs under the influence of chemokines (chemokine receptor CCR7),[33] and then undergo apoptosis or active

killing by cytotoxic T cells.[33] In the context of inflammation, DC stimulation, maturation and activation may initiate T cell pro-inflammatory cytokine production, cytotoxic function and B cell antibody production.[33]

Increased expression of co-stimulatory and adhesion molecules is characteristic of DC maturation and leads to efficient antigen presentation to T cells.[34] The DC maturation program itself is signalled by several pathways: NF-κB, MAPK and the Janus kinase-Signal Transducers and Activators of Transcription [(Jak-STAT), especially Jak3 and STAT4].[34] Finally, increased numbers of myeloid DCs that express T-cell costimulatory molecules (CD80, CD86 and CD40) have been found in synovial fluid of patients with RA.[35]

T cells

Following antigen presentation by DCs and APCs; activation of T cells is thought to be the next step orchestrating further events in the pathogenesis of RA. T cell activation following antigen presentation in association with co-stimulatory molecules, results in avid binding of long duration between the APC and T cell. One of the important receptor-ligand interactions involves CD40 ligand, which is a cell-surface molecule on activated T cells. CD40 ligand is essential for T cell-induced antibody formation by B cells and for causing APCs to induce cell mediated immune responses. CD40 on B cells and DCs interacts with CD40 ligand on T cells and results in up-regulation of CD80 and CD86 on DCs and B cells. When CD80 and CD86 interact with CD28 on T cells, T cell activation results.[36] Absence of the second co-stimulatory signal results in either poor activation or apoptosis. CTLA-4 (cytotoxic T lymphocyte associated antigen 4) expression occurs on the T cell surface following activation; this serves as an immunoregulatory protein that downregulates T cell activation.[37] Inhibition of co-stimulation by the monoclonal antibody, abatacept, has been successfully applied in the treatment of RA. Intriguingly, an excess of co-stimulatory molecules is present within rheumatoid tissue, and suggests the presence of T cell activation without a specific antigen. This might result in self-perpetuating cycles of T cell proliferation sufficient to sustain autoimmunity.[38]

Naïve T cells develop into effector T cells upon antigen recognition, while the cytokine milieu they encounter during development influences lineage specificity. RA has been long thought to be a Th1 mediated autoimmune disease because of the abundance of Th1 cytokines [especially interferon (IFN)-γ], the relative lack of Th2 cytokines (IL-4, IL-5, IL-

10), and the ability of Th1 cells to activate macrophages. This paradigm has been challenged by recent insights into T cell differentiation and its role in the pathogenesis of RA.

In 2005, the discovery of a lineage of T cells (Th17 cells) distinct from Th1/Th2 cells[39,40] and the subsequent recognition of IL-17's role in inflammation and autoimmunity[41] led to a re-evaluation of the understanding of the role of T cells in the pathogenesis of RA. Th17 cells produce several distinctive cytokines including IL-17A, IL-17F, IL-22 and IL-21 and express chemokine receptors CCR4 and CCR6. IL-17A is a pro-inflammatory cytokine that mediates its activities through a heterodimeric receptor complex composed of IL-17RA and IL-17RC subunits.[42,43] IL-17F is also pro-inflammatory but less so than IL-17A; its mechanism of action is similar to IL-17A.[42] Patients with RA have increased numbers of Th17 cells in the peripheral circulation, synovial fluid and the synovial membrane and higher IL-17A levels have been associated with RA disease severity.[42] The key cytokines for the development of human Th17 cells are transforming growth factor-β (TGF-β) plus IL-6, IL-21 and IL-1 followed by IL-23. In the presence of IL-1B and IL-23, or IL-6 and TGF- β in the presence of IL-21 or IL-23, Th17 cells undergo differentiation and express a unique transcription factor, ROR-c, which induces transcription of the IL-17 gene and subsequent IL-17 production.[44] Most parenchymal cells have IL-17 receptors, and signalling through these receptors induces target cells to produce several pro-inflammatory mediators including IL-1β, TNF and IL-6 and induces chemokines (IL-8 that helps recruit neutrophils and CCL20 that recruits $CCR6^+$ cells including DCs and Th17 cells).[42]

For example, on exposure to IL-17, macrophages produce IL-1, TNF and IL-6 causing inflammation; osteoblasts express the receptor activator of nuclear factor-κB (RANK) ligand (RANKL) which can activate osteoclasts leading to bone erosion.[44] Indeed, in RA, production of TNF, IL-1 and IL-17 by synovial cells is predictive of joint destruction.[45] IL-17 increases IL-6 production and IL-6 activates a positive feedback loop committing more naïve T cells to the Th17 lineage. Furthermore, Th17 cells induce chemokine production and attract numerous T cells into the inflamed tissue, amplifying the inflammatory cascade leading to further tissue damage.[44] Cells (including non-T cells) other than Th17 cells can produce IL-17. Anti-IL-17 therapy has been evaluated in clinical trials in RA but has not been found to be highly effective underscoring the complexity and redundancy of feedback loops and inflammatory pathways in the context of RA.

In addition to promoting cytokine production and the events described above, CD4+ effector T cells provide crucial help to B cells for antibody production and perpetuation of the autoimmune inflammatory cascade.

B cells

The role of B cells in RA is multifaceted and includes antigen presentation, cytokine production (IL-6, TNF-α, IL-10), modulation of T cell response and autoantibody production.[7] The presence of ACPA years before the onset of clinical disease implicates autoantigen specific B cells and plasma cell differentiation in the pathogenesis of RA.

B cells may function as APCs within the RA synovium, in addition to DCs and macrophages, by taking up antigen via surface immunoglobulin and efficiently processing and present it to T cells loaded onto class II MHC. In addition, B cells contribute to ectopic lymphoneogenesis (development of tertiary lymphoid tissue) with germinal centre (GC) like structures within the inflamed synovium in RA. Further maturation in these structures leads to differentiated memory B cells that can secrete cytokines including LT-α (lymphotoxin-α) which further promotes lymphoneogenesis, TNF that promotes inflammation and plasma cells that secrete RF and ACPA.[46] Activated B cells in the RA synovium express activation-induced cytidine deaminase (AID), an important enzyme for the initiation of affinity maturation events.[46]

In addition, evidence suggests that T cell activation in the RA synovium is also B cell dependant. [47] B cells may also play a role in bone homeostasis in RA as ectopic lymphoid follicles not only occur at other sites of inflammation in RA (e.g. lungs) but also in the subcortical bone marrow adjacent to the joint. Indeed, synovial CXCL3 (a B cell chemoattractant associated with extra-nodal lymphoid aggregates), has been found to be increased in severe, ACPA positive RA.[48] Certain subsets of memory B cells can also express RANKL and some express osteoprotegerin (OPG, a soluble decoy receptor of RANKL and an inhibitor of osteoclastogenesis), suggesting a hitherto underappreciated role of B cells in bone homeostasis in RA. [46] Finally, B cell depletion, using anti-CD20 antibodies, is an effective therapy for RA. [49]

Synovial lining fibroblasts, macrophages and mast cells

Both synovial fibroblasts (SF) and resident synovial macrophages play an important role in sustaining and perpetuating the inflammatory process [50] .

SF contribute to local inflammation and cartilage damage by producing pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs). In RA, SF undergo only limited apoptosis possibly owing to increased p53 tumour suppressor gene mutations in the inflamed synovium and consequent loss of function;[51] they assume a near-autonomous, aggressive phenotype and promote lymphocyte organisation and survival.[52-54]

Activated synovial macrophages are multipotent effector cells that integrate innate and adaptive immune responses very efficiently. They play a pivotal role in maintaining the chronic inflammation of RA. They abound at the cartilage pannus junction and exhibit strong phagocytic activity, antigen processing and presentation, secretion of pro-inflammatory cytokines, expression of Fc receptors that are auto-antibody and immune complex responsive, and play an important role in TLR signalling, complement activation, tissue degradation and remodelling and directly interact with fibroblasts and T cells.[55,56]

Mast cells may be important and possibly key players in the erosive and inflammatory events leading to joint destruction. Once activated, they release an exceptionally broad range of potent effectors including histamine, heparin, proteinases, cytokines, prostaglandins and growth factors, leading to changes in the microenvironment and contributing to inflammation and also playing a vital role in (neo)angiogenesis.[57-60]

The pathogenesis of RA in an individual patient: moving goal posts?

In summary, despite extensive research, the pathogenesis of RA remains poorly understood. Recent advances include elucidation of the molecular basis of the association of the SE with RA, further characterisation of the role of ACPA in the activation of autoreactive T cells and identification and the role of Th17 cells and IL-17 in RA. It is also now recognised that these inflammatory triggers are likely to change with time and many of the initiating processes are likely to occur before the onset of clinical disease. The variations in pathology in early versus late disease are significant and likely to impact upon therapeutic targets and hence response to therapies. Assessment of disease must recognise these differing phases; ideal clinical measures of disease activity will need to encompass these different periods in the pathogenesis of disease and this may include biochemical, physical and histological markers.

RA can lead to rapid development of joint damage and significant long-term disability.[61] Over the last two decades, there has been a paradigm shift from using only monotherapy with conventional disease modifying anti rheumatic drugs (DMARDs) to using combination therapy with a treat-to-target approach and finally now aiming for remission using a combination of conventional and biologic DMARDs. Several biologic DMARDs (Table 2) are now available with diverse mechanisms of action; despite this, it is also now evident that despite their promise and high costs, remission is an elusive target for a significant proportion of patients with RA. Regardless, intensive therapy with DMARDs substantially improves disease activity, radiographic progression and physical function[62] and hence accurate assessment of disease activity and defining remission is critical. Several attempts to define remission in RA have been made since the original 1950 definition by Short and Bauer, who described it as a state where "the disease was inactive, the patients were asymptomatic, and examination of the joints was negative except for residual deformity." [63]

Since no single measure can capture all aspects of RA activity, various composite measures have been developed over the years (Table 1), starting with the ACR remission criteria in 1981,[64] followed by the Disease Activity Score involving 44 joints (DAS44) and the DAS involving a 28 joint count (DAS28).[65,66] More recently, two further composite disease indices- the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI)- have been proposed.[67,68] The most recent have been the 2011 ACR (American College of Rheumatology)/ EULAR (European League Against Rheumatism) proposed criteria for remission.[69]

Table 1. Some of the commonly used disease activity, response and remission criteria for RA. DA- disease activity. H- high, M- moderate, L- low, Rem- remission, PGA- patient global activity, EGA- evaluator global activity. CRP in mg/dl for SDAI; PGA and EGA measured on a 10cm visual analogue scale (VAS); GH- global health measured on a 100mm VAS; ACR- American College of Rheumatology; EULAR- European League Against Rheumatism

Criteria for measuring disease activity		
	Formula	Cut-offs
DAS ESR [70]	0.54 x √(Ritchie Articular Index) + 0.065 x SJC44 + 0.33 x $\log_{nat}$(ESR) + 0.0072 x GH	LDA <2.4 MDA <3.7
DAS CRP	0.54 x √(Ritchie) + 0.065 x SJC44 + 0.17 x $\log_{nat}$(CRP+1) + 0.0072 x GH + 0.45	HDA ≥ 3.7

DAS28ESR[70]	0.56 x √(TJC28) + 0.28 x √(SJC28) + 0.70 x $\log_{nat}$(ESR) + 0.014 x GH	LDA 2.6-3.2 MDA >3.2-5.1
DAS28CRP	0.56 x √(TJC28) + 0.28 x √(SJC28) + 0.36 x $\log_{nat}$(CRP+1) + 0.014 x GH + 0.96	HDA >5.1
SDAI [68]	SJC28 + TJC28 + PGA + EGA + CRP	LDA ≤ 11 MDA ≤ 26 HDA >26
CDAI [68]	SJC28 + TJC28 + PGA + EGA	LDA ≤ 10 MDA ≤ 22 HDA >22

Criteria for response and remission	
DAS [70]	Remission < 1.6
DAS28 [70]	Remission < 2.6
SDAI [68]	Remission ≤ 3.3
CDAI [68]	Remission ≤ 2.8
ACR 20/50/70 [71,72]	At least a 20/ 50/ 70% improvement in 1. SJC 2. TJC and three of the following five 1. PGA (e.g. by VAS) 2. EGA (e.g. by VAS) 3. Patient pain assessment (e.g. by VAS) 4. Functional disability (e.g. by HAQ) 5. Acute phase response (ESR or CRP)
ACR 1981 [64]	Remission: at least ≥5 of the following for at least 2 consecutive months: 1. Early morning stiffness ≤ 15 minutes 2. No fatigue 3. No joint pain (by history) 4. No joint swelling or tenderness or pain on motion 5. No soft tissue swelling in joints or tendon sheaths 6. ESR < 30 mm/ hour (females) or < 20 mm/ hour (males)
ACR/ EULAR 2011 [69]	Remission: • SDAI ≤ 3.3, OR

	All of the following • Swollen and tender joint counts each ≤ 1 • PGA (0-10 scale) ≤ 1 • CRP (in mg/ dl) ≤ 1
EULAR [73]	Good response • Decline in DAS28 >1.2 and DAS28 score <3.2 Moderate response • Decline in DAS28 >1.2 (without reaching DAS28 <3.2), OR a decline in DAS28 of 0.6-1.2, plus reaching at least moderate disease activity (DAS28 <5.1)

Table 2. Some of the currently used bDMARDs in RA.[74,75] DMARD- disease modifying anti-rheumatic drug; MAB- monoclonal antibody; IL- interleukin; CTLA4- cytotoxic T-lymphocyte associated protein 4

Biologic type	Drug	Mechanism of action
TNF inhibitor	Infliximab	Chimeric MAB: human IgG1 Fc region joined to variable region of mouse anti-TNFα antibody
	Etanercept	Soluble TNF-receptor fusion protein dimer of 2 recombinant p75 TNFα receptor proteins with each molecule linked to the Fc portion of human IgG1
	Adalimumab	Recombinant human IgG1 MAB
	Golimumab	Recombinant human IgG1 MAB specific for TNFα
	Certolizumab pegol	Pegylated humanised anti- TNFα Fab fragment
T-cell co-stimulation inhibitor	Abatacept	Recombinant fusion protein consisting of Fc domain of human IgG1 fused to extracellular domain of human CTLA-4
B-cell targeted therapy	Rituximab	Chimeric MAB to CD20
IL-6 inhibitor	Tocilizumab	Humanised anti-IL6 receptor MAB

ACR Criteria for Remission and Response to Therapy

These were first proposed by the ACR (then American Rheumatism Association) in 1981. [64] Since it was not specified in these criteria as to how to measure the variables, Prevoo *et al.* [76] suggested modifications which replaced the absence of joint pain on history by a visual analogue scale (VAS), no joint tenderness or pain on motion was fulfilled if no joint was scored painful (out of 53 joints), no soft tissue swelling in joints or tendon sheaths was fulfilled if no joint scored swollen out of 44 joints and fatigue was not measured. The criteria for morning stiffness and ESR remained unchanged.[76] The ACR criteria have been criticised because additional factors such as structural damage to joints may cause pain without necessarily reflecting disease activity and it would still be possible to fulfil disease criteria for remission despite having swollen joints.[77] Despite these limitations, the ACR criteria are viewed as reliable outcome measures.

The ACR response criteria (Table 1) are different from the ACR remission criteria in that remission is an assessment of disease activity at a specific point in time, whereas a response is a measure as to how the disease activity changes with time, and the ACR20%, 50% and 70% improvement criteria were developed to evaluate response to therapy.[71,72]

The Disease Activity Score (DAS), DAS involving 28 joints (DAS28), and EULAR response criteria

The DAS (Table 1) was proposed by EULAR in the early 1990s, and is a composite, single-point, absolute measure of disease activity. The original DAS involved 44 joints (and hence called DAS44 or simply DAS), and included the Ritchie Articular Index (a graded measure of joint tenderness).[78,79] It was unwieldy to use, even in the research setting and subsequently, the DAS28, a new and relatively more time efficient score was proposed. [65,66] While the DAS28 is much easier to use in the clinical and research setting, it has attracted a number of criticisms and concerns. By not requiring assessment of the ankle and feet, it may under-represent disease activity in the early stages of the disease, a critical period for obtaining disease control. [80-82] The DAS28 definition of remission (based on a score of <2.6) has also engendered controversy [83] and lower cut-off values have been proposed. [70] The acute phase reactants (CRP / ESR) heavily weigh in the final DAS28 score which may erroneously lower the DAS28 score in the face of objective evidence of ongoing joint activity, [84] especially as a

significant proportion of patients with RA can have normal ESR and CRP at presentation and during the course of otherwise active disease.[81]

Notwithstanding its limitations, the DAS28 has been extensively validated not only for routine clinical use, but also as an outcome measure in clinical trials. Both the DAS and DAS28 have been used in several clinical trials that showed the benefit of tight control in the treatment of RA.[62,65,85,86]

The EULAR response criteria (Table 1) can be applied using either the DAS or the DAS28, and incorporate not only the level of disease activity but also the extent of change.[65] These criteria have been well validated [73] and have been shown to be comparable to ACR criteria.[87]

The Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI)

The complexity and requirement of computational tools for calculating the DAS28 was the driving force behind development of the SDAI (Table 1), a simple numerical summation of the values of a derived set of variables of disease activity. [68] The CRP, as opposed to the ESR was chosen as the acute-phase reactant as it is less confounded by other factors and was thought to more precisely reflect disease activity. In contrast to the DAS28, the evaluator global assessment (EGA) was introduced into the score to integrate subjective and objective measures as a part of the evaluation. The patient global assessment (PGA) remains because the EGA is seldom used without the PGA and patients usually view their disease as being more active than their physicians.[68]

Subsequently the CDAI (Table 1) was developed as an abbreviated form of the SDAI, excluding CRP from the formula. The rationale behind developing the CDAI was to enable the physician to make immediate therapeutic decisions regarding intensification of therapy if laboratory data was not available, because the acute phase reactants correlate with each of the other variables and may not add importantly to a composite score.[68]

Both the SDAI and CDAI have been validated for use in clinical practice. SDAI remission criteria may be more stringent than the DAS28 remission criteria on the basis of a study which demonstrated less inflammation by power Doppler in patients who were in SDAI remission vs DAS28 remission.[88] Similarly CDAI remission in patients with RA has been

shown to have quality of life scores closer to the healthy population as compared to those who were in DAS28 remission.[89]

FDA remission criteria

The FDA remission criteria are thought to be the most stringent, as they require ACR remission in addition to radiographic arrest as defined by the Larsen or Sharp scores, for 6 months following cessation of all anti-rheumatic drugs. To date, no therapy has been able to fulfill these criteria [77] but their development reflects a change in the paradigm of treatment of RA. Low activity disease is no longer seen as the best attainable outcome.

The new ACR/ EULAR remission criteria

These criteria, developed jointly by a committee of the ACR and EULAR define remission in RA using either a Boolean definition or SDAI $\leq$ 3.3 (Table 1).[69] The committee recommended but did not mandate a full joint count. In addition, a clinic-based definition suggested omitting the CRP as this may not be always available in the clinic setting.[69]

Problems with current disease activity and remission criteria

Several of the current disease activity and remission criteria use abbreviated joint counts (particularly the DAS28, SDAI and CDAI) that omit the ankle and feet, a common site of involvement in early RA (ERA), at a time when other joints may be relatively unaffected, and inflammatory markers normal.[80-82] There is also relatively poor correlation between individual criteria,[82] and perhaps tighter control should, in an individual patient, consist of aiming for achieving remission defined by more than one criterion, in addition to imaging evidence of remission (discussed below).

ADDITIONAL OUTCOME MEASURES IN RA

Apart from the clinical outcome measures described above, other outcome measures employed in RA include patient-reported outcome measures, radiographic outcomes and outcomes at the synovial membrane level. Finally the overall impact of RA as a disease has an important bearing on work, an outcome measure that probably best reflects its societal consequences.

Patient-reported outcome (PRO) measures in RA

Several PRO measures are used in RA and include measures of function [the Health Assessment Questionnaire (HAQ), modified HAQ (mHAQ)], health related quality of life [Rheumatoid Arthritis Quality of Life (RAQoL), Short-Form 36 (SF-36)] and patient response to illness (the Arthritis Helplessness and Rheumatology Attitude Index), among others.

HAQ and mHAQ

The HAQ is probably the most commonly used and most robust PRO used in RA. It has been instrumental in engendering a shift from clinical and biochemical parameters of disease assessments to outcomes that are more relevant to the patient. [90-92] The original HAQ was one of the first PROs to be published, and assesses multiple dimensions. [92] These dimensions are underpinned by patient-centred values that contribute to the HAQ's hierarchical structure and include avoidance of long-term disability, freedom from pain, avoidance of treatment related adverse events (AEs), containment of medical costs and postponement of death. While the full version of the HAQ includes all these values, the version that is most commonly used is an abbreviated version that includes the HAQ Disability Index (HAQ-DI), the HAQ visual analogue (VAS) pain scale and the VAS global health scale.[90]

The HAQ-DI includes 20 questions and covers 8 functional activities-of-daily-living categories: dressing, rising, eating, walking, hygiene, reach, grip and usual activities. These questions encompass fine movements of the upper limbs, locomotion and activities that involve both upper and lower limbs and takes into account use of aids of devices for assistance, as well as help from another person.[90]

The HAQ correlates well with clinical (joint counts) and laboratory (inflammatory marker) measures, and with physical capacity measures. In the context of RA, the main determinants of the HAQ are disease activity, pain and psychosocial factors rather than structural abnormality. [93] It is one of the strongest predictors of mortality, work disability and economic loss. [61,94]

The mHAQ is an abbreviated form of the HAQ, and uses only 8 questions. Despite its relative brevity and the absence of questions about aids or assistive devices, it has been found to closely correlate with the HAQ. However, it lacks a normal distribution, and fails to detect numerical improvement in scores (despite clinical improvement) in up to a quarter of patients; this floor effect has been shown to improve with addition of items.[95,96]

A further two modifications of the HAQ were developed: the multi-dimensional HAQ (MD HAQ), and the HAQ-II. The MDHAQ is another abbreviated version of the HAQ, includes more items than the mHAQ, has questions pertaining to demanding physical activity, pain, fatigue, anxiety and depression and avoids the floor effect in patients with limited disability. [95,97] The HAQ-II is also a shorter version of the HAQ, and like the MDHAQ, attempts to correct the floor effect seen with the mHAQ; it includes 5 items from the original HAQ and 5 additional items, with no subscales.[95,97] Of all the versions of the original HAQ, the HAQ-II has been shown to have the greatest uniformity between values over a longer scale than the HAQ, and skipped items impact least on the total score.[98]

RAQoL (Rheumatoid Arthritis Quality of Life) Questionnaire

The RAQoL was designed simultaneously in the Netherlands and the UK as a RA-specific quality of life instrument, and the final questionnaire consists of 30 questions that assess specific activities of daily living and quality of life.[99,100] The RAQoL is validated in Dutch, British, Swedish and Australian[101] populations, among others. It has been shown to have high internal consistency, test-retest reliability and discriminant sensitivity; it correlates well with the HAQ, DAS, joint counts and modified Sharp score.[102,103] In addition, physical contact, a dimension not covered by other common instruments in RA, is encompassed by this questionnaire. This is particularly relevant for patients with RA who are likely to have concerns relating to avoidance of shaking hands or being touched. [103] As an index of minimally important worsening, an increase of 2.0 on the RAQoL corresponds to an increase of 0.25 on the HAQ. [95,104]

SF-36

The SF-36 questionnaire originated from the Medical Outcomes Study. [105] It assesses multiple health concepts: limitations in physical, social or usual activities because of physical or emotional problems or pain, questions to assess energy, fatigue and general health perceptions, [106] and has been cross-culturally adapted and translated.[107,108] This questionnaire has been extensively used as a QoL outcome measure in RA [109-119] and as an instrument in assessing comprehensive disease control. [111] It has shown to respond to treatments and to distinguish therapies that translate to meaningful health benefits.[112] The mental health subscale may be less responsive to minimal clinical response [120] and the physical function component has been criticised for not assessing dexterity, which is commonly affected in

RA.[97] Similar to the HAQ, the SF-36 may overestimate disease activity in the context of concomitant fibromyalgia.[116]

Arthritis Helplessness Index (AHI) and the Rheumatology Attitudes Index (RAI)

The AHI and the conceptually very similar RAI are based on the learned helplessness (LH; in which participants believe their efforts will be ineffective) theory and were designed to assess patients' perceptions of loss of control with RA.[121] There are several variants of these questionnaires: an original 15 item AHI and RAI, and a 5 item helplessness subscale of the AHI and RAI. [121-123] Although the AHI and RAI have reasonable internal consistency, and the 5-item scale has a stronger correlation with health variables and is faster and easier to use, these scales suffer from relatively low reliability and it is recommended that they not be used as sole measures for clinical decision making.[124,125] LH has also been found to mediate the relationship between socio economic status and disease outcome, and to correlate with depressed mood in RA.[126,127]

Imaging outcomes

Plain radiographs of the hands and feet have been used in the evaluation of the course of RA for over six decades. The efficacy of synthetic and biologic DMARDs has been evaluated by their ability to slow or prevent radiographic damage. [128] More recently, ultrasound (US) and MRI have been employed as more sensitive measures to assess disease activity and progression.

Conventional radiography

Several radiographic scoring methods have been described over the past decades. They range from methods that provide a global assessment to those that assess individual joints. Probably the first established methods were the Steinbrocker and Kellgren methods.[129,130] These determined the extent of joint damage as a global score that was a summation of abnormalities of several joints and dominated by the worst change in any particular joint assessed in the hands. A consistent later trend has been to assess individual joints and assign a score to each joint; this commenced with the score proposed by Sharp et al in 1971, which correlated with clinical disease activity and was reproducible. Sharp's score assessed joint space narrowing (JSN), erosions, ankylosis, defects, cystic changes, periosteal reaction, cortical thinning, osteopenia, sclerosis and osteophyte changes.[131,132] A subsequent

modification of this score in 1985 saw the omission of periosteal reaction, cortical thinning, osteopenia, sclerosis and osteophyte changes because of technical and other issues.

There were several subsequent modifications [133-135] but it continued to include only the hands, until the van der Heijde modification (to include the feet) in 1989.[136] van der Heijde further modified this method by simplifying the scoring using the Simple Erosion Narrowing Score (SENS). [137] This score, which is probably superior to the Larsen method (see below) at the individual patient level, [138] remains widespread in use and includes 15 areas from the hands and wrists and 6 from the feet, with a maximum erosion score of 160 for the hands, 120 for the feet and maximum JSN 120 for the hands and 48 for the feet. The total score ranges from 0 to 448.[139]

An alternative method is that of Larsen, which was first proposed in 1974; the score was developed after a patient with RA, and a maximum Steinbrocker damage score of 4, was observed running for a bus.[128] It provided an overall index of joint damage (this method combined erosion and JSN as a single score; it reports on hands as well as feet) and differentiated stages of damage from normal (0) to 5. Again, this score has undergone several modifications, the most recent in 1995 and 1998.[140-143]

Although radiographs have been long considered to be the most durable indicator of disease progression, they are relatively insensitive to change, fail to detect early disease and underestimate joint damage; another major limitation is their inability to assess disease activity at a point in time or short term response to treatment.[144-148]

Ultrasound (US)

Of increasing importance in RA disease assessment, US has the unique advantage of being a point-of-care modality. As compared to conventional radiography, in ERA, US detected 6.5 fold more erosions in ERA (as compared to 3.4 fold in late disease). [145] It is at least as sensitive as MRI in detecting erosive disease and in very ERA can detect tenosynovitis, a precursor of more established RA. [144,149,150] In addition, it can detect joint effusions, synovial hypertrophy and hyperemia; US can evaluate effects of therapy and US detected erosions and power Doppler positivity have been shown to correlate with long term clinical and radiographic outcomes. [151-153] There have been attempts to evaluate and validate scoring systems that assess limited number of joints by US, as representative of disease activity. [151]

This imaging modality has the advantages of:

1. lack of exposure to radiation,
2. three-dimensional viewing,
3. reproducibility,
4. ability to image periarticular structures and adjoining soft tissues and
5. it is the only technique to reliably quantify bone marrow oedema.

Disadvantages include operator dependence, the need for contrast to reliably detect synovitis, inability to use in patients with ferromagnetic implants, high cost and limited accessibility.[154]

In the context of ERA, MRI can be very useful in detecting subclinical synovitis, early erosions, tenosynovitis and bone marrow oedema. Bone marrow oedema is probably the best test for RA diagnosis[155] and an early and reliable predictor of long term joint damage.[156] Flexor tenosynovitis as detected by MRI scan has been shown to predict RA. [157] MRI is more sensitive than conventional radiographs and this is illustrated by the fact that erosions are visible on MRI a median of 2 years prior to being detected by radiography; indeed it is only after 20-30% of the bone is eroded on MRI, that it erosions are detected on radiography.[158]

Under the auspices of OMERACT, the first RA-MRI score (RAMRIS) was developed in 2002, and erosion, bone marrow oedema and synovial volume were deemed to be the most reproducible measurements.[159] While specific to the hand and wrist, it has been expanded to include the feet, and it has been applied to measure bone volume.[160,161] Simplified methods for MRI reading have been developed,[162] and MRI is being increasingly used as an outcome measure in clinical trials in RA.[163] Cost and availability remain factors limiting its use.

Work ability as an outcome measure of RA

RA has profound societal consequences, with the largest proportion of societal costs being driven by inability to work. Historical data indicate that a significant proportion of patients with RA cease work. Work loss tends to happen early and worsen over time; a third of patients are unable to work within the first 5 years after diagnosis,[164] and more than half cease work after a decade, with 90% stopping work prior to retirement age.[165] Although there seems to have been a general decline in the rates of work disability from 50% at 10 years in the 1980s[165] to 35% at 10 years more recently[166] (in part, methodological differences between

these studies may account for the difference), these rates still remain unacceptably high. Work disability in RA may be explained on the basis of biomedical (i.e. disease activity and structural damage leading to functional limitation) or a biopsychosocial perspective (i.e. a misfit between functional ability and work demands).[167] It is now recognised that work outcomes relate only in part to disease activity and response to therapy.[165,168] Systematic reviews have identified a robust association with increasing age, functional disability, physically demanding occupations and lower education levels which are predictive of work disability; association with disease activity, structural damage and seropositivity remains inconsistent.[167,169]

Work disability in ERA

One of the first studies of work disability in ERA was that of Barrett *et al.* [164] Work disability was measured by a structured postal questionnaire in the Norfolk Arthritis Register. This included two primary care based inception cohorts with recruitment between 1989-92 (mean follow up 8.6 years) and 1994-97 (mean follow up 4.1 years). The rates of work disability for cohort 1 at 1, 2, 5 and 10 years were 14, 26, 33, 39% respectively; despite more aggressive treatment (no details of treatment given) in the newer cohort, rates of work disability were generally similar (23 and 33% respectively at year 1 and 2). The baseline HAQ was the most important predictor of ability to work.

Work outcomes were also analysed in the Finnish RA Combination Therapy (FIN-RACo) inception cohort that compared outcomes with combination (n=80; methotrexate, sulfasalazine, hydroxychloroquine and low dose prednisolone) vs. monotherapy (n=82; sulfasalazine only; prednisolone at clinician's discretion). The median follow up was 5 years. The duration of work disability per patient-observation year was significantly lower in the combination therapy arm (12.4 days) than in the monotherapy arm (32.2 days). This was mainly due to differences in median days per patient observation year of sick leave of 11.7 vs. 30 in combination vs. single therapy treatment groups. Furthermore, fewer patients in the combination treatment group (21%) were on disability pension at 5 years than in the monotherapy group (34%). [170]

Work outcomes have been assessed in relatively early DMARD naïve RA (<2 years; recruitment 1986-1998) in the ERA study (ERAS; median follow up 10 years). [171] Of the 647 in paid work at baseline, 245 ceased work because of RA (these patients were found to have

more severe RA). The probability of stopping work was highest in those older (45-60 years) at diagnosis.

In the ERA Network (ERAN) inception cohort (n=1235; median follow up 3 years; RA managed according to local guidelines), 47% (475) were employed at baseline. During the follow up period, there was loss of employment in 10% and of these, over half reported loss of employment secondary to RA. Disease activity, pain, smoking and poor mental health were associated with earlier work disability while a better EULAR response was associated with a lower probability of claiming new health benefits. Of those who lost their job, 84% (41/49) retired and only 5 returned to work.[172]

Biologic DMARDs and work disability in ERA

The advent of biologic agents and the greater prospect of remission as a therapeutic goal has engendered interest in work as an outcome measure in RA clinical trials. [173]

A cross sectional study from Sweden included 3029 patients (treated with conventional and biologic DMARDs) with ERA (<12 months; median follow up 3 years). [168] At baseline, the mean number of sick leave days in each group were 13 and 44., 26% and 30% had full (0 work days lost the month before), partial (1-29 work days lost) and no (≥ 30 work days lost) work ability. At 3 years, 71% patients with full baseline work ability were working compared to 36% and 18% of those with partial and no work ability at baseline. The best predictor of work ability was baseline ability to work. Other predictors were HAQ, DAS28, age and education level. Neither ESR nor CRP was predictive of work ability.

A study [174] that included methotrexate inadequate responders who had either placebo (n=282) or the TNF inhibitor, infliximab, (n=722) add on therapy to methotrexate, found that patients treated with methotrexate and infliximab in combination had greater likelihood of employability (defined as either working or able to work if work was available) and less workdays lost. Similar outcomes were noted in other studies that compared methotrexate to methotrexate/TNF inhibitor combination. [175-177]

The seemingly beneficial effect of bDMARDs in terms of work ability may reflect inadequacy of treatment and higher disease activity in the methotrexate only arm, rather than a true effect of biologic DMARDs. This was elegantly addressed in a well-executed clinical study, the SWEFOT study. [178] This study randomised patients who had not achieved low

disease activity after 3-4 months of methotrexate only therapy to methotrexate in combination with sulfasalazine plus hydroxychloroquine or infliximab. Monthly sick leave and disability pension days were retrieved 21 months after randomisation. Mean changes in work lost were 4.9 days/month in the biologic and 6.2 days/month in conventional treatment groups. Although there was some radiographic superiority of the biologic treated group, this did not translate into better work outcomes.

Evidence for efficacy of a treat-to-target strategy and improved work outcomes

It is accepted that a treat-to-target approach leads to better clinical outcomes in RA.[62,179] In terms of influencing ability to work, there is a limited body of evidence to support this, the most prominent among them being the aforementioned work outcomes from the FIN-RACo and SWEFOT studies.[170,178] There is also limited evidence of maintenance of work capacity in treated-to-target patients as compared to loss of working hours in the general population. The ability to modify work-related factors may be limited and hence treatment options that optimise chances of remission may have a significant impact in reducing work disability.

Synovial biopsy and changes in the synovial membrane as outcome measures

Synovial biopsy is a low risk procedure that can provide valuable macroscopic and microscopic information about an individual's disease[180,181] in addition to traditional parameters**.** Indeed, biopsy proven synovitis precedes clinically manifest arthritis in ERA,[182] and synovial biopsy may yield histological and molecular markers to identify patients with a poor outcome, provide a sensitive means of assessing response to treatment, or identify patients who are likely to respond to a particular treatment option. [183-186]

Methods of synovial biopsy

The synovium is amenable to biopsy by arthroscopy or by using blind needle or ultrasound directed techniques.[183] Blind needle techniques have been established for decades and have a good safety and feasibility record.[187] They can be undertaken in an office setting, are relatively low-cost, and do not require special facilities. The major concerns of blind biopsy techniques lie in failure to obtain satisfactory samples, especially if the joint is clinically quiescent because of failure to visualize involved areas; in addition the joints that are amenable to biopsy by this technique are limited. [187] In one series with more than 800 Parker-Pearson biopsy procedures, sufficient tissue was obtained in about 85% of patients for histological examination and no haemarthroses or infections occurred.[188] The authors found

that the biopsy was most likely to fail in joints that were not swollen and unfortunately, this failure rate is unacceptable within the context of "proof of concept" phase IB or II RCT where arthroscopic synovial biopsy is acceptable. [187]

Arthroscopic synovial biopsy has the advantage of macroscopic examination, visually directed biopsies with better sampling from areas of interest, its major disadvantages being the need for a "learning curve" and the requirement for operation theatre facilities.[187] In addition, arthroscopic biopsies may more accurately estimate the degree of inflammation as sampling from sites adjacent to cartilage, which usually display a higher degree of inflammatory changes, is possible. This area is difficult to access by needle biopsy[183] Complication rates with arthroscopies performed by rheumatologists are similar to those reported in the orthopaedic literature: in a study evaluating 16 532 arthroscopies in which 50.5% and 49.5% of the arthroscopies had a clinical and research indication respectively revealed a complication rate of joint infection in 0.1%, wound infection in 0.1%, haemarthrosis in 0.9%, deep venous thrombosis in 0.2% and neurological damage, thrombophlebitis and other complications in 0.02%, 0.08% and 0.06% respectively. Irrigation volume correlated with wound infection rate and centres that performed cartilage biopsy had a higher rate of haemarthrosis. [181]

Macroscopic appearance of the synovium in RA

Macroscopically, the synovium in RA has a distinct vascularity pattern with straight vessels (as opposed to tortuous vessels or a mixed pattern in spondyloarthritides, reactive arthritis and psoriatic arthritis). The macroscopic appearance may predict histological changes (albeit with only moderate correlation) and clinical parameters, with the straight pattern portending a worse outcome. However, there is no widely accepted scoring system or well-validated method of description of macroscopic changes, and no reliable method to predict microscopic features, especially in an individual patient.[183,189,190]

Inter- and intra-articular variation in synovial membrane pathology and biopsy in involved *vs.* uninvolved joints

The only study to address the issue of whether biopsy from an involved joint is representative of the synovial infiltrate in other involved joints was a study of 9 patients with established RA. This study compared (by immunohistochemical digital image analysis) the cell infiltrate in paired synovial biopsy samples from inflamed knee joints and paired inflamed small joint (wrist or MCP), and found no significant differences in mean cell numbers of T cells,

sublining macrophages, plasma cells and IL-6 expression. There was no significant correlation between different joints for the number of intimal macrophages or fibroblast like synoviocytes.[191]

Several studies have addressed the issue of intra-articular variation in synovial infiltrate from different areas of the same joint. The first study, comparing arthroscopically directed vs. blind needle biopsy samples, found greater numbers of macrophages in biopsies of synovium adjacent to cartilage, and a good correlation (between arthroscopic vs. blind biopsy samples) was found for macrophages in the lining and T cells in the sublining. [192] In another study, arthroscopically obtained paired synovial biopsy specimens from cartilage pannus junction (CPJ) and suprapatellar pouch (SPP) from knee joints of 17 patients with RA found generally similar features with regards to T cells, macrophages, plasma cells and expression of MMP (especially MMP3).[193] A third study of 8 patients with RA analysed synovial membrane (SM) specimens from the CPJ and an area remote to the CPJ and found greater macrophage infiltration and higher expression of myeloid related proteins at the CPJ.[194] In general, there does not appear to be a significant difference between tissue from the CPJ vs non-CPJ in terms of features of synovial inflammation and mediators of inflammation and destruction.[187,195,196]

There have been several studies that have examined the variability of SM measures from a single joint (both within the same biopsy and between multiple biopsy specimens from the same joint). A study in which 145 synovial biopsy specimens from 30 procedures performed on knee joints of 29 patients with DMARD-naïve active RA revealed considerable homogeneity in a single joint for intensity of synovial lining layer hyperplasia, vessel proliferation, mononuclear cell infiltration and fibrosis.[197] In another study of 8 patients with RA, needle arthroscopic biopsies were taken from multiple sites around a knee joint and quantification of immunohistochemical staining was done by colour video image analysis. No difference between intra-biopsy and inter-biopsy variability for cell adhesion molecule staining and limited variation in cytokine expression in the sublining and vessels was found.[198] A third study which analysed between- patient, between-biopsy and intra-biopsy variation in RANKL and OPG staining (both important in RA) by immunohistochemistry (IHC) in synovial tissue from patients with active RA found marked variability for RANKL expression (probably secondary to variability in T cell infiltration), with OPG expression being more consistent inter and intra-biopsy than between patients.[199] In general, the

variability found in SM biopsy analyses probably reflects the biological variability of expression. This suggests that while restricting the number of samples examined histologically may improve feasibility it would sacrifice reliability.[187] In particular for T cell infiltration and expression of activation antigens in the RA synovium, a small study found that <10% variance can be reached when at least 6 biopsy specimens are examined.[200]

Analysis of synovial biopsy specimens in RA

Synovial histology provides the most direct window into the inflammatory cascade driving RA in the individual. In conjunction with IHC and molecular analyses, it correlates with disease activity,[183,201] predicts response to treatment,[202-207] and can prognosticate with regards to disease outcome.[184,208]

Quantification of inflammation by histology and IHC

The histological assessment of synovium in RA includes intimal thickness and density and composition of the cellular infiltrate. [183] The normal synovium has a lining layer that is 1-2 cells thick, consisting of Type A synoviocytes (macrophages) and Type B synoviocytes (SF).[209,210]

Three methods of quantification of the cellular infiltrate, cell phenotype, cell surface receptor expression and cellular adhesion molecule expression have been used in published studies: manual counting (MC), semiquantitative scoring (SQA) and digital image analysis (DIA).[187]

MC, the "time honoured gold standard" is done with the help of a graticule and is a valid measure when the variable measured is localized to a cell; there is less certainty with regards to the validity of this method when non cell associated variables (such as cytokines) are measured. It is subject to observer bias, though reliability has been documented between observers in the same research laboratory. There has been no published inter-laboratory testing.[187]

SQA, a grading of biopsy staining at low to medium power magnification, eliminates field selection bias and is the fastest of all the techniques. There remains some observer bias, though intra- and inter-observer reliability has been demonstrated.[187]

DIA is the newest technique that has been validated with regards to inter- and intra-observer reliability for the widest range of biological variables including cytokines, MMPs, vascular markers, adhesion molecules and chemokines. It is also the most expensive, and has a significant learning curve. Results are expressed as the area of staining (in pixels), density of staining (in units) or as a combined measure (integrated optical density or IOD, in pixel units). DIA has a potential for observer bias in selection of thresholds and field selection, but is probably faster than MC and for this reason, has become more widely adopted [187,189]

Gene expression profiling (GEP) and microarrays

For GEP on synovial biopsy samples, a validated real-time quantitative polymerase chain reaction (Q-PCR) can be used. It is recommended that ≥ 6 synovial tissue samples be used to limit sampling error. This method, developed to complement IHC uses a cell-based standard curve generated with cDNA derived from activated peripheral blood monocytes.[189] Expression microarrays offer the potential for analysis of all genes in synovial tissue.[189] These devices contain large numbers (tens of thousands) of short DNA probes of specified sequences arrayed in an orderly fashion and attached to a flat surface. Each spot on the array corresponds to a particular probe which itself corresponds to a gene, to which RNA derived from that gene may bind.[211] In RA, analysis of expression profiles with a focus on immune-related genes in synovial tissue has revealed considerable variability with identification of molecularly distinct forms of the disease.[3,212]

Another approach to GEP on whole tissue samples is examination of specific areas in tissue sections isolated by microbeam laser microscope and subsequently subjected to nested RNA arbitrarily primed-polymerase chain reaction (RAP-PCR) for differential display fingerprinting.[213] Although technically difficult, this has the benefit of targeting only the cell type of interest.

The synovium in ERA

Vascular changes in early synovitis

Microvascular changes occur very early in the course of the disease with vascular congestion and obliteration being prominent findings. In RA the synovial blood vessels are relatively straight in contrast to the tortuous and bushy appearance seen in early psoriatic and spondyloarthropathy (SpA).[214]

Cellular changes

As early as four to six weeks following the onset of symptoms of RA, synovial lining layer thickening of up to 10 cells is noted, along with vascular proliferation and diffuse subintimal inflammatory infiltrate consisting of macrophages, lymphocytes, neutrophils, mast cells and DCs. [209] Synovial fibroblast proliferation and macrophage recruitment are both thought to be contributors to thickening of the synovial lining [215] and are of particular importance as they are both implicated in engendering joint damage.[52,216,217] In keeping with this fact, radiological erosions have been shown to occur early in the disease, often within the first 2 years, usually initially in the feet and even before joint space narrowing.[218-220] Importantly, cellular changes in joints are well-established before the clinical onset of disease[182] and asymptomatic joints also show cellular changes, mainly in the form of lining layer hyperplasia, though to a lesser degree than in clinically involved joints.[221] Also, in early disease, the majority of cellular subtypes including monocytes, macrophages, lymphocytes and plasma cells, usually thought typical of established RA, are present.[214,222] The degree of lymphocytic, plasma cell and neutrophil infiltration is similar between ERA (when defined as <12 months) and established RA,[214] and at least in one study, was independent of disease duration.[201] In contrast, lymphoid follicles appear to generally be a feature of late and not ERA.[214]

A study by Kottinen *et al* [223] evaluated patients with acute (<3 months, n=4), subacute (well established chronic RA with no clinical signs/ symptoms in the knee joint examined, n=4) and chronic (n=5) RA. They found a predominance of monocytes/ macrophages and patchy T cell infiltration in acute arthritis as opposed to perivascular T cells and sublining B and plasma cells, in addition to monocytes. In chronic synovitis, there was a shift to prominent T cell and plasma cell infiltrates. Subsequent studies have also been limited by small numbers and it is unsurprising that in this setting their findings were replicated only in some series. [224]

In another study [225] which looked at 15 patients with ERA (disease duration <6 months), a synovial membrane perivascular lymphocyte infiltrate, including lymphoid follicles with germinal centres with a close interdependence in these lesions of CD20cy (CD20 being the intra-cytoplasmic epitope of CD20) and CD45RO cells was found.

Overall, conclusive evidence for a distinct histological pattern in ERA is lacking, and the differences observed may relate not to disease duration but how active the disease was at the

time of sampling. This was illustrated nicely in a well-designed study[226] that included 16 patients with ERA [and similar numbers with late RA, early SpA and osteoarthritis (OA)]. In this study except for maximal synovial lining thickness, no difference was found between early and late RA, though differences were found between RA and SpA/ OA.

Also, given the established changes in "early" RA, it is worthwhile noting that "early" RA is a clinical and not a histological definition and changes at the synovial level precede clinical symptoms by an indeterminate period of time.[224]

Cellular adhesion molecules

Inflammatory cells are recruited to the synovium by interacting with cell adhesion molecules (CAMs) on leucocytes and synovial vascular endothelium.[209,227,228] CAM expression in ERA and established RA and OA has been compared. In ERA, intercellular adhesion molecule 1 (ICAM1) was found throughout the synovium, E-selectin, P-selectin and very-late-antigen1 (VLA1) were mainly found on endothelial cells, vascular cell adhesion molecule (VCAM) 1 on lining layer cells (E-selectin, ICAM1 and vascular cell adhesion molecule or VCAM facilitate leucocyte adherence to the vessel wall), platelet-endothelial cell adhesion molecule (PECAM1; this facilitates subsequent migration into tissues) predominantly on endothelial cells, but also in the lining layer, sublining layer and on infiltrating cells. Integrins αvβ3 and αvβ5 were found on the lining layer and endothelial cells; sublining cells expressed αvβ5. It was found that expression of these adhesion molecules in ERA was similar to that of established RA, but greater than in OA. [214,229]

Cadherin 11 is known to be increased in the presence of inflammation, is driven in part by TNFα and to modulate MMP production;[227,230] in synergy with TNF-α and IL-1β is particularly relevant in inducing SF to secrete pro-inflammatory cytokines, including IL-6. [231] Studies on cadherin-11 in ERA are sparse, but limited evidence suggests downregulation with prednisolone therapy; there was no significant difference in cadherin-11 expression in inflammatory synovium of RA, SpA or inflammatory OA. [230] In summary, markers of endothelial activation are prominent in both ERA and established RA, and the subtle differences in inflammatory cell infiltrate seen cannot be explained on the basis of adhesion molecules alone. [214]

Cytokines and chemokines

A study of SM from 31 patients with ERA and 35 with established (>5years) RA, found similar expression of interleukin-6 (IL-6) and TNFα. [201] Another study [232] that included patients with relatively ERA (<18 months) found wide variation in cytokine expression between patients despite similar macroscopic and histological features of inflammation. IL-1α and IL-1β had the most prominent expression albeit at different sites (IL-1α mainly in endothelial cells and IL-1β on synovial lining cells); there was less TNFα expression compared to IL-1 expressing cells; when TNFα was present it tended to be greater in pannus tissue rather than in synovial villi.

In a study [228] that compared chemokine expression in ERA (n=22, duration of disease <12 months) and established RA (n=22, duration of disease >5years) there was CD68+ macrophage accumulation and as expected, expression of MIP 1α and MCP1 were observed in both ERA and established RA, but there was a further increase in CD68+ macrophage infiltration and MIP1α in the synovial lining layer in the established RA group. CD68 expression correlated with MIP 1α but not MCP1 expression. In another study, [233] CXCL 8 (IL-8) was also found in ERA and reduced following treatment with methylprednisolone. Chemokines bind to chemokine receptors, of which CCR2 and CCR5 (both receptors of the CC family of chemokines) have been demonstrated on synovial fluid cells in established RA, [209,234] as has increased expression of fractalkine (CX_3CL1) which has been shown to stimulate angiogenesis. [235]

A seminal paper by Raza *et al.* [236] evaluated synovial fluid aspirates from inflamed joints of early inflammatory arthritis patients (disease duration <3 months), whose outcomes were determined subsequently. As comparators, synovial fluid was aspirated from patients with crystal arthritis, established RA and OA. Twenty-three cytokines and chemokines were assessed in the synovial fluid. It was found that patients destined to develop RA had a distinct but transient synovial fluid cytokine profile. In those patients who went on to develop RA, the levels of a range of T cell, macrophage and stromal cell related cytokines [including IL-2, IL-4, IL-13, IL-17, IL-15, basic fibroblast growth factor (FGF) and epidermal growth factor (EGF)] were significantly elevated within 3 months of symptom onset as compared to those who did not develop RA. This profile was not present in patients with established RA. The Th2 cytokine pattern (IL-4, IL-13) was an unexpected finding in ERA, as the current paradigm suggests RA is a Th1 [236] (or perhaps a Th17) [237] driven disease. The authors

suggested that this distinct cytokine response is likely to influence the microenvironment required for persistent RA;[236] in any case these important findings lend pathophysiological credence to the observed so-called "window of opportunity" with regards to treatment of ERA.[238-240]

Another study [237] that compared cytokines related to neutrophil and Th17 activation in very ERA (VERA; disease duration <6 weeks, n=38 of which 19 fulfilled the 1987 ACR criteria for RA), healthy donors (n=27), established RA (n=15) and OA (n=10) found that patients with VERA had increased serum levels of cytokines promoting Th17 polarization (IL-1β and IL-6) as well as IL-8 and Th17 derived cytokines (IL-17A and IL-22). Synovial fluid samples were not available from the VERA group and the authors noted that this Th17 pattern was more evident in the synovial fluid than in serum of established RA patients.

Neutral protease enzymes and their inhibitors

Serine proteases (elastase, cathepsins and granzymes) capable of degrading collagen and proteoglycan molecules and MMPs are largely the culprits for cartilage and connective tissue destruction in the RA synovium that finally leads to loss of joint integrity.[209] Granzyme expression by natural killer (NK) and T cells has been demonstrated to be greater in ERA than in established RA in keeping with the observation that joint damage occurs early in the disease.[209,241] mRNA for cathepsin B and cathepsin L has been found in both ERA and established RA, with cathepsin L possibly expressed at a higher level in ERA.[184]

There are 4 classes of MMPs (collagenases, gelatinases, stromelysins and membrane bound or MT MMPs) that can be distinguished on the basis of their substrate specificity.[209] The gelatinases (MMP2 and MMP9) have been associated with tissue invasion and metastatic disease in the oncology context, and in patients with inflammatory arthritis, these are expressed in the SM and have been implicated in the invasion of synovial tissue into adjacent cartilage and bone, apart from having a role in angiogenesis.[242,243] In a study[242] of 66 patients with synovitis of <1 year duration (of which finally 45 fulfilled the 1987 ACR criteria for RA), MMP2 was widely expressed in the synovial lining layer and in areas of stromal proliferation in the sublining layer and stroma of the 12 patients who developed erosions. MMP9 was expressed more sparsely and focally. MMP14 (the activator for pro-MMP2) was significantly higher in RA, in contrast to TIMP2 which was lower. [242]

Of the MT-MMPs, MT1-MMP has been observed at sites of bone resorption and MT3-MMP in the SM in established RA. [244] The TIMPs (tissue inhibitors of MMPs) are physiological inhibitors of MMPs of which 4 (TIMP1-4) and both TIMP1 and TIMP2 have been demonstrated in ERA; in a study that compared patients that achieved ACR remission vs. those who did not, those that achieved ACR remission showed reduction in MMP-1 and MMP-3 and corresponding increase in TIMP-1 and TIMP-2 with reduction in the MMP:TIMP ratio.[242,245,246]

Toll-like receptors and small molecules

In concert with synovial macrophages, activated SFs contribute to cartilage and bone destruction; one of the strong stimulators of RASFs are Toll-like receptors (TLRs). [247] TLR signalling causes co-stimulatory molecule up regulation by APCs which then facilitates adaptive immune responses by providing a second signal to T cell stimulation. [247]

It has been shown that TLRs 2, 3 and 4 are expressed in the synovium of patients with long standing RA and stimulation via the TLR2 pathway leads to translocation of NFκB, secretion of pro-inflammatory cytokines and expression of chemokines. Similarly, stimulation of TLR3 and TLR 4 pathways by endogenous (or synthetic) ligands induces interferon-β, IL-6 and the chemokines CXCL10 and CCL5.[247-251] A recent study in patients with ERA (disease duration <12 months), revealed high expression of TLRs in SF, especially TLR 3 and TLR 4 along with reactivity of SFs *in vitro*, suggesting that TLR signalling pathways are activated early in the disease and eventually contribute to persistent inflammation and joint destruction.[247] SF expression of TLRs (especially TLR 3) is increased by IL-17, and TLRs in turn perpetuate Th1 and Th17 responses in RA. [252,253]

Apoptosis in the synovial membrane in ERA

A failure of apoptotic pathways might be the explanation for the SM hyperplasia, angiogenesis and mononuclear infiltrates seen in RA synovial tissue.[254] In a study[255] that compared apoptosis by TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end labelling) in SM biopsy specimens from 11 patients with established RA and 8 with ERA (mean duration 5 months), there was limited apoptosis in the ERA as compared to established RA. They also found that the number of macrophages and FLIP (FLICE like inhibitory protein that inhibits death receptor mediated apoptosis) was higher in ERA, and postulated

that there is defective apoptosis early in the disease that is restored later as the disease progresses.[255]

Another regulator of apoptosis is the p53 tumour suppressor gene. In the context of RA, while p53 expression has not been found to be different in ERA *vs.* established RA, p53 gene expression was detected in the lining layer, endothelium, lymphocytic aggregates (where present), and diffuse leukocytic infiltrates of inflamed synovium;[256] as mentioned earlier, these are probably loss-of-function mutant transcripts that prevent apoptosis.[51] In addition, endothelial cell apoptosis has also been found to be greater in ERA, as compared to early psoriatic arthritis (PsA) patients.[257]

Gene expression profiling and molecular signatures

In a study of 20 patients (12 of whom had ERA) who were subject to closed needle synovial biopsy or needle arthroscopy, expression of MMP1, cathepsin B and cathepsin L were examined by in situ hybridization. Of those who developed erosions over the course of 1 year, MMP1, cathepsin B and cathepsin L mRNA in the synovial lining, perivascularly and in the endothelial cells of the sublining were higher as compared to those who did not.[184]

In a more recent study using microarray analysis[258] in which arthroscopically obtained synovial biopsies of 15 patients (4 untreated ERA, 4 treated long standing RA and 7 controls) were obtained and subject to large scale gene-expression profiling, and found different gene-expression combinations in ERA, long standing RA and controls, suggesting the involvement of different pathophysiological mechanisms during the course of RA.

Individualising therapy based on synovial biopsy findings should improve outcomes in ERA

In summary, although synovial findings are variable and depend not only on the duration of the disease but also disease activity at a point in time, the synovium provides a wealth of information in an individual patient, not otherwise usually obtainable, with considerable reliability and relatively minimal trauma and we postulate that the differential synovial responses to therapy make synovial biopsy an attractive option to use in the context of personalised medicine. The particular time points this might be most relevant are probably at baseline, and at the times of both excellent and poor therapeutic response, as a loss of response over time is well known and is not yet fully explained, and biopsies at these time

points have the potential to be hugely informative in terms of directing the next therapy, including biologic DMARD therapy.

PREDICTORS OF RESPONSE TO TREATMENT IN RA

The approach to treatment of RA has changed significantly over the past two decades and achievement of minimal disease activity or preferably remission is the desired goal for all patients. To minimise disability, early loss of work, and also to bring the disease under control during the treatment window period when better outcomes are obtained, it is important to maximise our chances of obtaining initial, good response to treatment. Despite our advances in therapy, our ability to predict patients that will response to a particular therapy, either conventional or biologic, remains limited. The next section reviews evidence in this area.

Predictors of treatment response to conventional DMARDs

With few exceptions, most research in terms of predictors of response to therapy has been in established RA rather than ERA. The most common DMARD studied in terms of prediction of response has been methotrexate, with some data for combination therapy and sulfasalazine. Data on predictors of response to other DMARDs are sparse.

Demographic and other factors

In DMARD-naïve ERA patients, factors associated with a poor prognosis to methotrexate in the SWEFOT study were current smoking, female sex, longer symptom duration and younger age. [259] Other factors known to be associated with a poor response are RF, high pain score, patient global assessment and disability scores.[260-262]

Genetic markers

The most abundant source of genetic variation in humans is the single nucleotide polymorphisms (SNPs) which occur every few hundred bases in promoter regions and coding and non-coding sequences. Most allelic variations are located in the intergenic regions and hence thought to be of no consequence, however function SNPs can alter promoter activity (regulatory SNPs), DNA, pre-mRNA conformation, or mature RNA (alternate splicing) and by doing so, can alter the function or expression of the gene product (the protein). SNPs may therefore play a direct or indirect role in the demonstration of the therapeutic response.[263]

For methotrexate (Mtx), genetic polymorphisms that affect its uptake or efflux are of interest. Indeed the disposition properties of Mtx may be influenced by polymorphisms in the reduced folate carrier (RFC) gene which is responsible for cellular uptake and the P-gp (P-glycoprotein, MDR1 gene) thought to facilitate cellular efflux. [263] Mtx is taken up by cells using the RFC followed by polyglutamation via folate polyglutamate synthase. Mtx polyglutamates (MTXPG) are thought to be primarily responsible for the therapeutic effects of Mtx. Mtx inhibits enzymes of the folate pathway including dihydrofolate reductase, thymidilate synthetase (TS) and aminoimidazole carboxamide ribonucleotide transformylase (ATIC). A lower pharmacogenetic index (which comprised of polymorphisms in RFC, ATIC and TS) was associated with a poor response to Mtx.[264]

Sulfasalazine (SSz) has been difficult to study as its exact mechanism of action is yet to be elucidated which restricts selection of candidate genes. Polymorphisms in the N-acetyl transferase (NAT) 2 gene (which codes for the first enzyme in the metabolic pathway of SSz) have been identified which may influence inter-patient response to SSz.[263]

Genetic polymorphisms in the folate pathway were reviewed in a relatively recent[265] clinic-based study in 98 patients with RA (including ERA, diagnosis based on the 1987 ACR criteria) who received combination therapy with Mtx (known to exert its effects through the folate pathway among other putative mechanisms), SSz (known to inhibit enzymes of the folate pathway) and hydroxychloroquine (HCQ; no known effect on the folate pathway). The authors found polymorphic variations in the MTR, SLC19A1 and TYMS genes were associated with a better clinical response to combination DMARD regimens containing Mtx and SSz.

The shared epitope (SE) allelles have been one of the most commonly looked at genetic markers in relation to treatment response. O'Dell and colleagues reported the SE positive patients treated with combination DMARD therapy did much better than with Mtx alone; SE negative patients did equally well regardless of combination or monotherapy.[266] An older prospective study evaluated variables predicting response to gold and found that HLA-A3 positivity and HLA-DR4 negativity were the best predictors of the response to gold.[267]

It is known that leflunomide causes reduced production of IL-1β, IL-6 and TNFα, however a study that examined polymorphisms in IL1B, IL6 and TNF genes found no statistically significant relationship between genetic polymorphisms involving these genes and response

to leflunomide treatment. [268] A study that analysed circulating cytokines and regulatory T cells (Tregs) in RA of variable duration found that patients who responded well to corticosteroids had higher levels of TGFβ, Foxp3 and Tregs and lower levels of TNFα and IL-17.[269]

Leflunomide works by inhibiting dihydroorotate dehydrogenase (DHODH), and variants of DHODH haplotype may influence efficacy; [270] on the other hand despite biological plausibility, PTPN22 polymorphisms (known to lead to altered T cell responses) were not associated with leflunomide response.[271]

At this time, testing for genetic polymorphisms is not usually performed prior to therapy, although with the advent of personalised pharmacotherapy, this may eventually play a role in therapeutic decision making in RA.

Serum markers

A recent prospective study [272] in patients with active, but not necessarily ERA with DMARD treatment chosen according to the treating rheumatologist's decision, evaluated P-gp and several cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, TNFα) and found serum TNF to be a predictor of response to DMARDs. A small study [273] (n=13) by Gerli *et al.* found that patients with ERA who responded better to DMARD therapy (as measured by a fall in CRP) had higher basal serum CD30 (a member of the tumour necrosis/ nerve growth factor receptor superfamily) as compared to non-responders. In another study [274] of 50 patients (active but again not necessarily ERA) good (ACR 50-70) or excellent (ACR>70) responses to Mtx treatment were seen in those with a lower ratio of IL-1ra/ IL-1β constitutively produced by peripheral blood monocytes (PBMC) compared to patients with a poor (ACR<20) response.

In the FIN-RACo trial (which looked at combination DMARD vs DMARD monotherapy plus prednisolone in RA), a serum IL-2 level <442 units/ml at baseline was an independent predictor of remission at 6 months regardless of treatment regimen, sex, RF status, ESR and TJC/ SJC.[275]

Synovial markers

While there have been several studies that have documented changes in the synovial membrane with conventional DMARD treatment, literature on synovial markers predictive of DMARD response, is limited.

Predictors of treatment response to bDMARDs

The bDMARDs have involved some of the largest clinical trials of RA allowing assessment of markers of response in a more homogenous population. Their prohibitive cost has also driven research to identify those most likely to benefit from early treatment.

Demographic and other factors

Duration of disease, age of onset, baseline DAS28, presence of rheumatoid nodules and number of radiographic erosions did not correlate with TNFα inhibitor response in RA.[276] However high disease activity or smoking status were markers of poor response as well as female sex; concurrent methotrexate therapy was associated with improved response.[276-278]

Genetic markers and proteomics

Inheritance of a double dose of the SE has been found to predict response to etanercept; these patients were 3-4 times more likely to achieve an ACR50 response after 12 months of treatment with etanercept as compared with methotrexate.[279] TNFα and LTA (lymphotoxin alpha) loci are arranged in the class III HLA region between HLA-B and HLA-DR genes on chromosome 6, an area known to be highly polymorphic with several SNPs. Studies have been conflicting in terms of whether there is an association of the TNFA-308GG, TNFA-138GG and TNFA-857GG genotype with a better response to anti-TNFα agents. [276] A recent study analyzing genetic variants within the p38 MAP kinase signalling network found seven SNPs in 5 genes associated with improvement in the DAS28 at 6 months with infliximab and adalimumab but not etanercept.[280]

A proteomic approach by Trocme et al [281] in which plasma profiles of 60 patients with RA were analysed found higher apolipoprotein A-1 in responders and higher platelet factor 4 (PF4) in non-responders to infliximab. Another proteomic approach found higher serum MCP-1 and EGF as predictors of a good response to etanercept. [282] None of these approaches have been validated in larger cohorts.[276]

Serum markers

Anti-TNFα agents

Most commonly used markers including RF, CCP and CRP had no definite or a heterogenous association with therapeutic response to anti-TNFα agents. [276] The value of serum RANKL and OPG as predictive markers of therapeutic response to anti-TNFα agents (infliximab and

adalimumab) was investigated by a Spanish group who found that serum RANKL and RANKL/OPG ratio in patients who achieved remission were significantly lower at baseline and that lower serum RANKL levels were independently associated with a better response. [275] Although several serum biomarkers have been demonstrated to change with anti-TNFα agent treatment, including TNFα, IL-1β, IL-6, IL-10, IFN-γ, markers of bone remodeling, synovial activity and cartilage breakdown, their baseline levels have failed to predict a response to anti-TNFα agents.[276,283-286]

Rituximab

The serum markers that predict a better response to rituximab include higher ACPA titres, [287] lower levels of IFNγ and B-cell activating factor (BAFF) and a favourable FcγRIII genotype. [207] Interestingly in a study (double blind phase IIa trial of 161 patients randomized to Mtx, rituximab, rituximab plus Mtx or rituximab plus cyclophosphamide) B cell depletion was shown to have no relationship with clinical response. In addition, the time taken by B cells to recover was variable and did not predict rituximab responders. [288] In another study, [289] 60 established RA who had previously failed anti TNFα therapy received rituximab and the reconstitution of their B cell population was carefully monitored. B cell numbers were assessed by a highly sensitive flow cytometry prior to each infusion and 3 monthly thereafter. The best outcomes were in those who had complete B cell depletion, with poor outcomes in those who had a complete lack of B cell depletion. Patients who were depleted only after the second infusion did not do better than those who had a complete lack of depletion.

Nakou *et al*. [290] studied the effect of rituximab in peripheral blood (PB) and bone marrow (BM) B and T cell populations in active established RA, and found that rituximab preferentially depletes activated CD19+HLADR+B cells in the PB and BM and that clinical response to rituximab was associated with depletion of CD19+CD27+ memory B cells in PB and BM in RA. Vital *et al.*[291] evaluated the level of pre-plasma cells, memory and naïve B cells at baseline and post rituximab and found that initial non-responders to rituximab had higher circulating plasma cell counts and had incomplete depletion of B cells.

Interestingly, rituximab also depletes CD20+ T cells (usually constitute ~1.6-2.4% of T cells) in the PB and while this may be an additional mechanism of action of rituximab, it is not known if this is a marker predictive of response. [292]

Tocilizumab

There is some evidence that IL-6 receptor polymorphisms may be associated with response to tocilizumab.[293]

Synovial markers

Anti-TNFα agents

There have been no synovial tissue studies in DMARD naïve ERA patients. In a study by Buch *et al.* [294] of 51 patients with established RA who had failed at least 2 DMARDs prior to infliximab therapy, pre-treatment synovial TNFα, IL-1α and IL-1β expression (by IHC) did not differ between those who achieved an ACR response and the non-responders. Interestingly neither the baseline nor post- infliximab TNFα expression (TNFα expression was reduced in both groups following infliximab treatment) was different between ACR responders and non-responders. A study by Wijbrandts *et al* [295] included 143 patients with active established RA (all had failed at least 2 DMARDs). All patients had synovial biopsies prior to infliximab treatment. Baseline synovial TNFα expression and the number of synovial tissue resident and infiltrating macrophages were associated with a clinical response after 16 weeks of infliximab therapy, in contrast to the study by Buch *et al*. None of the several other synovial parameters studied (SF, T-cells, B cells, plasma cells, IL-6, IL-10, ICAM-1, VCAM, E-selectin, VEGF, basic fibroblast growth factor) were predictive of a response to infliximab.

Another study[296] of 97 patients with active established RA was undertaken to address whether the presence of lymphocyte aggregates in the synovium prior to infliximab treatment could serve as a biomarker for response. These patients had failed a mean of 2 DMARDs prior to synovial biopsy. Lymphoid aggregates were found in 57% of patients at baseline, 32% of which had large aggregates. Aggregates were found in 67% of clinical responders (by DAS28 and EULAR) as compared with 38% of non-responders. When factored into a prediction model that included baseline DAS28, anti-CCP positivity and synovial tissue TNFα expression, positivity for lymphoid aggregates was a significant predictor of clinical response.

Another study[185] that included 18 patients with active established RA (failed at least 2 DMARDs) had arthroscopic synovial biopsies pre-infliximab followed by large scale gene expression profiling using microarrays. The 12 patients who responded to infliximab had up-regulation of several biological processes related to inflammation (including T cell mediated

immunity, cell surface receptor mediated signal transduction, MHC II mediated immunity, cell adhesion, cytokine and chemokine mediated signaling pathway, etc.) compared to those who did not respond to treatment.

Badot et al [297] analysed the gene expression profile in paired synovial biopsies from affected knees of 25 DMARD resistant established RA patients at baseline and 12 weeks of adalimumab therapy and identified 439 genes associated with a poor response to therapy. A majority of these genes were upregulated in poor responders and classified into two specific pathways of cell division and regulation of immune response by cytokines and chemokines and their receptors.

Abatacept

In a study [298] of 16 patients with established RA who had previously failed TNFα therapy and then went on to abatacept, synovial biopsies post-abatacept therapy revealed a small reduction in synovial B cells with quantitative PCR showing reduction in expression of inflammatory genes. There have been no studies in ERA.

Rituximab

Studies on the synovium in the context of rituximab therapy have shown that synovial B cells are depleted but not completely eliminated by rituximab therapy, with greater depletion of B cells and reduction of CD68+ synovial macrophages correlating with clinical response.[180,207,299,300] A study[206] that analysed paired (at 0 and 3 months; n=27) synovial biopsy samples of TNF inhibitor non-responsive patients treated with rituximab, found no baseline differences in terms of clinical or IHC characteristics that could predict remission at 6 months. However analysis of specific molecular signature by gene expression studies revealed upregulation of immunoglobulin genes and genes involved in antigen processing and presentation via class II MHC as predictive of response. Further IHC revealed more Igκ light chains in responders.

Tocilizumab

A recent study[301] that analysed paired (at 0 and 3 months) synovial biopsy samples of DMARD naïve ERA patients treated with tocilizumab (n=17) or methotrexate (n=13) found no baseline differences in terms of clinical or IHC characteristics that could predict remission at 6 months. However analysis of specific molecular signature by gene expression studies

revealed overexpression of genes involved in Ras protein signal transduction and cell cycle pathways in the tocilizumab treatment group and overexpression of transcripts in myeloid cell function in methotrexate treatment group as predictive of SDAI remission at 6 months.

Another recent study[302] analysed synovial samples (histology, IHC, microarrays) from RA patients undergoing arthroplasty/synovectomy of affected joints (2 cohorts, n=49 and n=20) and serum samples from the ADACTA (tocilizumab vs. adalimumab; n=198) study. Four major synovial phenotypes (lymphoid, myeloid, low inflammatory, and fibroid) were found; the gene signatures for each were different. Those with myeloid gene expression pattern and elevated baseline serum soluble ICAM1 responded better to anti-TNF therapy; IL-6 response showed the opposite relationship to these biomarkers.

Predicting response to treatment on the basis of synovial biopsy findings in an individual patient

These synovial studies are intriguing and provide interesting insights into the factors that may predict a response to specific DMARD therapies. However further extrapolation to identify predictors for RA patients at large is limited by the fact that these studies focussed on markers that would allow prediction of response to a specific bDMARD agent, often in those who had failed multiple other therapies. Therefore, a review of the synovium as a whole was not undertaken. It is well recognised that most synovial biopsies from RA patients will show a selection of lymphoid aggregates, synovial proliferation and varying cytokine production, reflecting the complex inflammatory process. In this setting, perhaps the dominant inflammatory process in a particular patient may provide a more accurate target, and increase response rates.

RANKL, OPG, OSCAR and Dkk-1 as predictors of treatment response and predictors of bone damage in RA

Bone remodelling, the removal of old bone by osteoclasts and replacement by bone forming osteoblasts, is a lifelong spatially coordinated process. [303] The formation of an ‘osteon’ (the fundamental functional unit of bone) commences when as yet unknown signals attract osteoclast precursors (that are derived from the same bone marrow monocyte-macrophage lineage that give risk to macrophages and DCs) to a previously quiescent bone surface (activation phase), followed by fusion of osteoclasts to form multinucleated pro-osteoclasts that attach to bone surface, differentiate and start the process of bone resorption (resorptive phase). For osteoclast differentiation and activation, osteoblasts are obligatory by producing

factors that promote osteoclastogenesis as a response to overall regulation by bone resorbing hormones or cytokines. Apoptosis of osteoclasts then occurs, followed by a reversal phase in which preosteoblasts move to bone surface, differentiate into osteoblasts, and mediate new bone formation and mineralization (formation phase). The final phase of return of the bone to its previous quiescent phase occurs when there is apoptosis of osteoblasts (which then incorporate into bone as osteocytes or bone lining cells).[303] A delicate balance exists between bone formation and resorption; this balance is critical in maintaining bone health and in engendering disease.

RANKL, RANK and OPG

Receptor activator of nuclear factor κB ligand (RANKL) is one of two molecules (the other being macrophage colony stimulating factor or M-CSF), that is both mandatory and sufficient for osteoclastogenesis[304] and resultant bone resorption. RANKL is a tumour necrosis factor (TNF) family member that binds to its cognate receptor RANK (itself a homotrimeric TNF receptor family member) present on cells of the osteoclast lineage. RANK mediates all the known activities of RANKL. RANKL is essential for osteoclast formation, activity and survival, thereby exerting a major influence on bone turnover and remodelling in health and disease. RANKL acts by inducing the master regulator of osteoclast differentiation, NFATc1 (nuclear factor of activated T cells, cytoplasmic 1) by calcium calmodulin signalling mediated by a specific phosphatase, calcineurin. Phospholipase Cγ (PLCγ) is critical for this activation. PLCγ activation by RANK, in turn, requires the protein tyrosine kinase Syk, along with ITAM (immunoreceptor tyrosine-based activation motif) bearing molecules, such as DAP12 (DNAX- activating protein) and Fc receptor common gamma chain (FcRγ).[305]

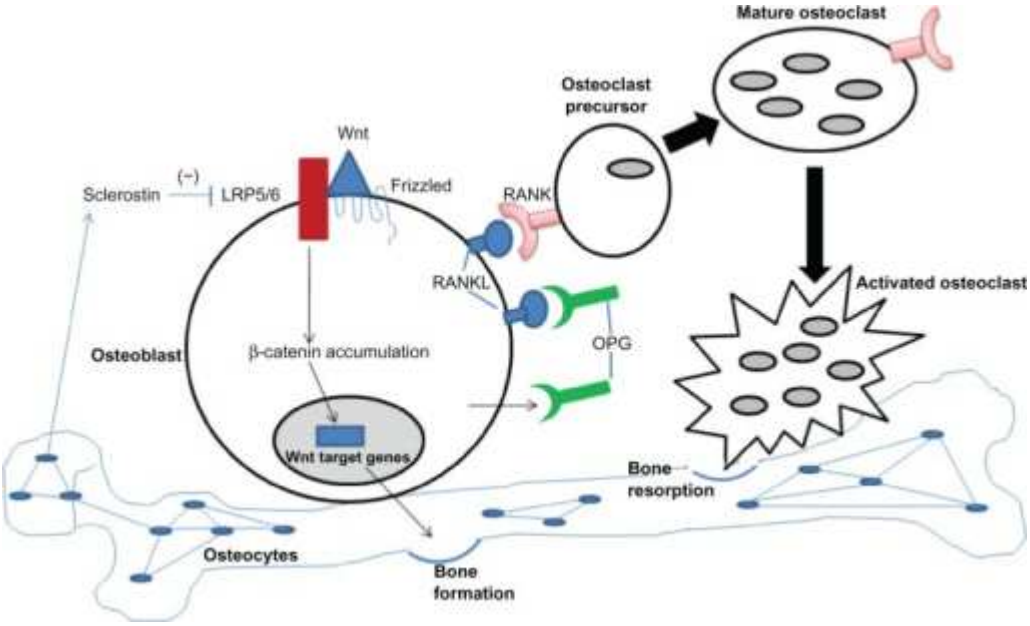
Osteoprotogerin [OPG, previously called osteoclastogenesis inhibitory factor (OCIF)] acts as a decoy receptor of RANKL. As a secreted protein with no transmembrane domain and no direct signalling properties, it is an atypical member of the TNF family. OPG prevents the interaction of RANKL with RANK and thus prevents osteoclast activation.[303]

The relative balance between RANKL and OPG is important in dictating the dominance of bone formation or resorption; the RANKL/RANK/OPG axis is now recognized as a central regulator of osteoclast differentiation and function.[306] A cartoon depicting RANKL/OPG and the related Wnt pathway (described below) is presented in **Figure 1**.

The major biological sources of OPG that regulate bone metabolism have not been established definitively. It is known that osteoblasts, cells of the osteoblastic lineage,

macrophage type synovial lining cells and endothelial cells among others (endothelial cells, stromal cells, etc.) produce OPG.[303,307,308] RANKL is produced by several cells including osteoblasts, activated T cells and SF.[303,308,309] RANKL is present as a functional membrane bound trimeric molecule from which a soluble homotrimeric molecule can be produced by activated T cells or generated from the membrane-bound form by the metalloproteinase disintegrin TNF alpha convertase (TACE).[309]

Figure 1. The RANKL/OPG/Wnt pathway in bone remodelling



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Link: http://openi.nlm.nih.gov/detailedresult.php?img=3796859_tacg-6-075Fig1&req=4
Link to Licence: https://creativecommons.org/licenses/by-nc/3.0/

OSCAR

Molecular overlap between other cells of the immune system and bone resorption is considerable. Indeed, of the several factors that regulate osteoclastogenesis (including M-CSF, RANKL, TGF-β, IL-1, TNF, among others), many also influence macrophages or DCs that share bone marrow precursors.[310] OSCAR (osteoclast-associated receptor), a co-stimulatory receptor expressed on osteoblasts, may help to explain why osteoclasts are bone-

specific.[311] This receptor is a member of the newly identified leukocyte receptor complex and associates with ITAMs on the common γ-chain of the Fc receptor. OSCAR-Fc has been shown to decrease osteoclast formation in pre-osteoclast:osteoblast co-cultures, indicating a putative osteoblast ligand.[305] RANKL-RANK induction of NFATc1 is crucial for OSCAR expression and ligand activated OSCAR interacts with FcRγ to increase intracellular calcium, thus augmenting NFATc1 expression and engendering a positive feedback loop that increases osteoclast mediated bone resorption.[312] OSCAR was also found to be expressed on monocytes, neutrophils and DCs and is involved in antigen presentation as well as survival, maturation and activation of DCs.[313] Indeed, ligation of OSCAR on monocytes and neutrophils induces a pro-inflammatory cascade, which may be of relevance in rheumatoid arthritis and immune mediated bone loss.[314]

Dkk-1

Dickkopf-1 (Dkk-1), serves as a functional inhibitor (but not the only one; the other being proteins of the WISE/ Sclerostin family) of the canonical Wnt/β-catenin pathway.[315,316] In the absence of Wnt, cytoplasmic β-catenin is degraded by the Axin complex. This complex is formed by the scaffolding protein Axin, the tumour suppressor adenomatous polyposis coli gene product, casein kinse 1 (CK1) and glycogen synthase kinase 3 (GSK3). β-catenin is degraded by CK1 and GSK3 mediated sequential phosphorylation, subsequent ubiquitination and proteasomal degradation. Wnt protein binding to cell surface co-receptor complex comprising of low-density lipoprotein (LRP)5/ LRP6 and the Frizzled (Fz) family of proteins leads to binding of the intra-cellular protein Dishevelled (Dsh) to Fz, LRP5/6 phosphorylation ensues and Axin is recruited thus disrupting Axin complex mediated β-catenin degradation. β-catenin accumulates, translocates to the nucleus, forms complexes with T-cell factor/ lymphocyte elongation factor family of transcription factors, and modulates gene expression.[316] The Wnt pathway is thought to contribute to bone formation by stimulating osteoblast differentiation and inhibiting osteoblast apoptosis, and by inhibiting osteoclastogenesis.[317] Dkk-1 binds to LRP5/6 and a co-receptor Kremen-1/2, promotes internalisation, followed by degradation of this complex, thus dampening the Wnt pathway.[316] The Wnt pathway and LRP5 have been found to be major regulators of bone mass and Dkk-1 to be the 'master regulator' of joint remodelling.[315,318] Levels of Dkk-1 are thought to be critical in determining whether an arthritic joint responds by erosion or bone formation, regardless of the presence of inflammation.[319] This effect led to confirmation of

cross-talk between the Dkk-1-RANKL-OPG systems and in experimental models, the anti-resorptive potential of anti-Dkk-1 monoclonal antibodies has been attributed to increased OPG expression, at least in part.[315,319]

RANKL, OPG, OSCAR and Dkk-1 in RA

In rheumatoid arthritis (RA), bone erosion has consistently remained a robust marker portending a worse outcome.[218,320,321] Serine proteases (elastase, cathepsins and granzymes) capable of degrading collagen and proteoglycan molecules and matrix metalloproteinases (MMPs) are the main mediators of cartilage and connective tissue destruction in the RA synovium, finally leading to loss of joint integrity.[209] Erosive disease characteristic of RA occurs at the cartilage pannus junction and osteoclast activation is thought to be an essential step in bone destruction.[308] Several inflammatory cytokines in RA synovial tissue [interleukin (IL)-1α, -1β and -6, TNFα and macrophage colony stimulating factor)] can potentially activate osteoclasts through up-regulation of RANKL (expressed by pre-osteoclasts, T cells and dendritic cells) and RANK, alter the RANKL/OPG ratio and cause bone resorption.[308,322,323]

RANKL and OPG have been the subject of several studies in RA including *in vitro*, synovial and other clinical studies.[203,308,324-327] In a study of 20 healthy adults and 20 patients with ERA, T cells and monocytes isolated from peripheral blood revealed surface RANKL, as did synovial fluid cells. Subsequent T cell/monocyte co-culture in patients resulted in osteoclast differentiation in patients but not in controls. This osteoclast differentiation was significantly inhibited by neutralizing monoclonal antibodies to TNFα, IL-15 and IL-17. [324] In another study of RANKL and OPG mRNA in peripheral blood mononuclear cells (PBMC) and synovial tissue in chronic RA, OA and healthy controls using polymerase chain reaction, elevated RANKL mRNA levels were found in patients with RA. The RANKL was shown to be mainly of CD4+T cell origin.[326] A study that evaluated the changes in RANK, RANKL and OPG on PBMC and co-cultured SF in the presence of varying doses of DMARDs found inhibition of osteoclastogenesis by inhibition of RANKL in SF by methotrexate, sulfasalazine and infliximab.[328]

Studies[203,308] that measured RANKL expression in the synovium in ERA (<12 months) found higher RANKL expression in active RA synovium compared with spondyloarthropathy. Subsequent DMARD therapy led to successful reduction in the RANKL:OPG ratio. Another study [309] examined OPG and soluble RANKL (sRANKL) in serum and synovial fluid

(measured with ELISA) in 44 patients with RA and 41 with OA; these levels were compared with clinical and radiologic scores. They found a positive correlation between sRANKL and synovial RANKL in the OA group, but only a trend to positivity in the RA group. Serum and synovial OPG was lower in RA; synovial RANKL was higher in RA. A third study [308] found OPG expression on macrophage type synovial lining cells in RA patients with active synovitis. At the synovial level, successful DMARD treatment resulted in increase in OPG expression and reduction in RANKL level which correlated with reduction of erosion scores in the hands and feet. [203] Another study [329] found high RANKL/OPG ratio in pannus tissue in patients with RA as compared to OA. A study evaluating the effect of anti TNF therapy in RA found increased expression of synovial OPG post therapy with a reduced RANKL/OPG ratio. [330]

In a study of 92 (of 155) patients from the COBRA cohort,[331,332] the RANKL/OPG ratio as measured at baseline independently predicted the 5 year radiographic progression of joint damage (as scored by the Sharp/ van der Heijde method), along with the time averaged ESR. [327] This cohort was subsequently followed up (with a total of 155 patients) and over 11 years. Unfavourable baseline levels of RANKL/OPG ratio in patients with early active untreated RA (at baseline) were found to be strong predictors of rapid and persistent progression of radiologic damage.[325] Osteoclasts are known to be essential for TNFα mediated joint destruction [333] and another study that looked at OPG and RANKL in longstanding severe RA treated with anti TNF agents at baseline and after anti TNF agent therapy found lower levels of sRANKL and RANKL/ OPG ratio as predictors of remission.[334] A study on fish oil supplementation in RA found increased levels of OPG and reduced levels of sRANKL and sRANKL/OPG ratio post therapy. [335]

In contrast to this, in a study [336] of patients treated with rituximab, [337] there was no significant change in sRANKL and OPG after therapy, though there was a significant decrease in the bone degradation marker desoxypyridinoline crosslinked collagen I. This provides credence to the fact that other mechanisms, not yet clearly elucidated, may exist that lead to bone protection, not directly involving measurable RANKL and OPG changes. Another study [338] looked at serial OPG, RANKL and osteopontin (OPN, a protein that is thought to help osteoclasts bind to bone [339]) in 25 patients with active RA who were randomised to receive either etanercept alone (n=13) or in combination with methotrexate (n=12). Levels of OPN, OPG, RANKL were reported at baseline, 4, 8, 12 and 16 weeks of treatment. Baseline levels

of OPN were significantly elevated compared to controls. sRANKL increased at 8 and 12 weeks in the monotherapy group. A significant reduction in the OPN level was observed in the etanercept group after 16 weeks of treatment.

There have been studies of OSCAR in RA. One of the first was by Herman *et al.* [314] who analysed OSCAR expression in the synovium and PBMCs in 17 TNF inhibitor treated patients with established RA. They also compared serum levels of OSCAR (sOSCAR) in these patients with age and gender matched controls. This study showed OSCAR expression by osteoclasts at the site of erosion and by mononuclear cells around synovial blood vessels, and higher levels of OSCAR on PBMCs of patients with RA. Higher OSCAR expression was correlated with higher disease activity. In contrast, sOSCAR were lower in RA as compared to healthy controls. Similar findings were reported in a Chinese study [340] involving 40 patients and age and gender matched healthy controls. In this study, sOSCAR was lower in RA. Furthermore, it was lower in active disease and erosive disease.

In contrast to these findings a recent cross sectional study[341] that compared sOSCAR in erosive ('destructive') vs. non-erosive RA and healthy controls found higher sOSCAR in RA, with higher levels in erosive RA vs. non-erosive RA. sOSCAR levels correlated positively with disease activity, inflammation and seropositivity. Further studies in another RA patient cohort, including longitudinal correlation with radiological outcomes are required to address the inconsistencies between these two studies.

A small (n=27) study of patients with established RA treated with methotrexate found lower Dkk-1 and higher OPG/RANKL ratio in those responding to therapy and higher than baseline DKK levels and lower OPG/RANKL ratio in those who were non-responders.[342] Bone marker analysis from the Torpedo study (n=163; this study was a study of clinical and ultrasound parameters in patients treated with tocilizumab in combination with methotrexate) revealed reduction of Dkk-1 following treatment at week 12 and week 48; levels of OPG and serum sclerostin showed no significant change.[343] Another study which measured only baseline Dkk-1 in 113 patients with RA (disease duration <3 years) treated with etanercept or methotrexate found an association of higher baseline Dkk-1 with risk of erosion progression in the etanercept but not methotrexate treated group.[344]

Prediction of erosive disease in the individual patient

There is a well-known dissociation between treatment response and progression of erosive disease and as yet there are few established markers that provide predictive information on

whether an individual is likely to go on to develop radiologic damage despite a response (of lack of response) to therapy. It is possible that the levels of RANKL, OPG, Dkk-1 and OSCAR may provide valuable information to guide therapeutic decisions.

TARGETED THERAPY IN RA

Despite an explosion in understanding underlying immunologic mechanisms and emergence of biologic therapies against cellular, cytokine and small-molecule targets, achieving sustained clinical remission in an individual patient using personalised therapy remains a distant goal. A key to the development of a targeted therapeutic approach is identifying therapeutic targets in an individual patient. A summary of these principles follows.

Cytokines as therapeutic targets

Several cytokine-based therapies have been successfully applied in RA: therapies against TNFα and IL-6 are now well established, although those against IL-1 have been disappointing despite strong pre-clinical rationale.

The first notable success was the identification of TNFα as a therapeutic target by Feldman and Maini. A pleiotropic cytokine, TNFα was identified in the synovium in the early 1980s and a chimeric mouse antibody was developed in the late 80s. [345] The first step in TNF production is ligand activation of a cell surface receptor on macrophages, DC and lymphocytes (and other cells), inducing NFκB activation and transcription of genes that induce TNFα (among other cytokines) production. This then recruits inflammatory cells to further release cytokines and augments the inflammatory response.[346] TNFα is present in a precursor transmembrane form; this is processed by metalloproteinases including TNFα converting enzyme to a soluble form that mediates its activities through Type 1 and Type 2 TNF receptors (TNF-R1 and TNF-R2); transmembrane TNFα acts as a ligand by binding to TNF-R as well as functioning as a receptor that transmits signals back into TNFα producing cells.[347] TNFα blockade was found to also inhibit other inflammatory cytokines including IL-1.[348] Subsequently, several other monoclonal antibodies were successfully developed targeting the membrane or soluble form of TNFα.

Although TNFα inhibitor treatment represented a very successful major advance in targeted therapy, it became apparent over time that this success was relative and although better responses were achieved in early disease, in established disease, 20% or less achieved

ACR70 responses, [349] with even lower rates following failure of a first TNFα inhibitor or failure of alternate biologic DMARDs. Nonetheless, TNFα inhibitor therapies are now well established in the treatment of RA.

A relatively recent cytokine that has been successfully targeted by biologic therapy has been IL-6, a spectacular example of bench to bedside research by Kishimoto and colleagues.[350] IL-6 is a pleiotropic cytokine produced by a variety of cells including T cells, B cells, fibroblasts, endothelial cells, and monocytes, among others. Although originally described as a T cell factor inducing B cell differentiation, it was later found to affect several other cell lines It induces synthesis of other cytokines, prostaglandins, metalloproteinases, activation of SFs and induces naïve T cells to differentiate into Th-17 cells. It is also central to the acute phase response. [350,351] IL-6 acts through a transmembrane IL-6 receptor, which is also found on multiple cells. The intracellular portion of the IL-6 receptor lacks a tyrosine kinase domain and IL-6 ligation on the cell surface causes this intracellular segment to associate with gp130. This segment subsequently dimerizes to activate JAK, which in turn induces tyrosine phosphorylation of STAT 3. STAT 3 translocates to the nucleus to induce gene expression. [351] Anti-IL-6 therapy with tocilizumab is now well established in the treatment of RA.

There are several additional cytokines emerging as therapeutic targets: these include granulocyte-monocyte colony-stimulating-factor (GM-CSF), IL-17, IL-20 and IL-21. GM-CSF acts by binding its receptor (GM-CSFR). A heterodimer composed of cytokine specific α and a shared beta chain, this receptor also mediates intra-cellular signalling via the JAK2-STAT3- STAT5 cascade. A recent successful trial of Mavrilimumab, a competitive antagonist of GM-CSF has been reported. [352]

IL-17 is a pleiotropic cytokine that acts through its receptor (IL-17R) which is present on several cell types including immune cells, SFs and epithelial cells. There are several members of the IL-17 family, of which IL-17A and IL-17F have been found to be up regulated in activated T cells and in RA (a more detailed description is provided earlier in this review). Trials of secukinumab and ixekizumab (monoclonal antibodies against IL-17A) have recently been reported [353,354] and phase III trials are underway.

IL-20 belongs to the IL-10 family of cytokines; IL-20 is expressed by monocyes and receptors have also been found to present in SF, higher levels of IL-20 are found in RA synovial fluid as well. IL-20 induces expression of IL-6 and promotes neutrophil chemotaxis,

SF migration and endothelial proliferation.[355,356] Recently, a phase IIa study showing benefit of an anti-IL-20 antibody in RA was reported.[357]

IL-21 is crucial for Th17 T cell maturation, and has been found to be increased in the serum of patients with RA; it is also associated with B cell activation, promotion of osteoclastogenesis and radiographic progression.[44,358-361] Phase I studies of anti-IL-21 mAb have been completed, and results are awaited.

Tyrosine kinases as therapeutic targets

Tyrosine kinases (TKs) are one of the two (the other being serine/ threonine kinases) protein kinases that are activated by extracellular signals (which include cytokines and growth factors). TKs transmit signals to the nucleus either by way of membrane receptors with intrinsic TK activity or through cytoplasmic or non-receptor TKs. [362] Most of the current therapeutic approaches have employed inhibition of non-receptor TKs; these include inhibition of JAK, SYK (Spleen TK) and BTK (Bruton TK). Previous approaches included inhibition of the serine/threonine MAP kinase pathways with disappointing results, despite biological plausibility.[363]

JAKs act through activation of STATs which then dimerize, migrate into the cytoplasm and translocate to the nucleus leading to transcription of target genes. The JAK family includes JAK1, JAK2, JAK3 and tyrosine kinase 2 (Tyk2); JAK activation occurs following cytokine binding to its unique cognate receptor (each JAK has its own cytokine receptor). Many cytokines important in inflammation in RA interact with JAKs, including IL-6 (described above), IL-2, -7, -15, -21, -27, -31, and IFN.[362] JAK inhibition with tofacitinib as mono- or add on therapy is now established in RA.

There has been recent interest in SYK; this TK has been found to have a role in IL-1β production through the NOD-like receptor protein 3 inflammasome. ITAM activation, triggered on engagement of an APC, activates SYK which then autophosphorylates and mediates downstream pathways including MAP kinases, NFAT and NFκB. Results of a phase II study of SYK inhibitors in RA have been reported. SYK inhibition was found to be effective, with neutropenia the major side effect (SYK is known to have a role in haematopoiesis).[364]

Another cytoplasmic TK of interest is BTK, which is recruited following antigen engagement of the B cell receptor, leading to effects on B cell mediated cell proliferation. BTK mutations are the cause of Bruton's agammaglobulinaemia, and BTK inhibition as a therapeutic approach is being investigated in RA.[362]

Cell based therapeutic targets

T cells and Treg cells

T lymphocytes have long been a potential therapeutic target in RA. Initial attempts with T cell depleting therapy using Campath-1H were met with limited success and unacceptable toxicity. More recently inhibition of T cell co-stimulation signals (described earlier in this review) has been successfully deployed in RA.

Recent interest has focussed on regulatory cells and it is now recognised that a number of cells including Treg cells, Breg cells, regulatory DCs, suppressive macrophages and CD8+ suppressor T cells may all play a role in inhibiting activated T cells.[365]

Treg cells suppress T-cell proliferation, and adoptive transfer of $CD4^{+}CD25^{+}$ Treg cells reduces arthritis severity in animal models.[366] Unfortunately, delineating Treg cell function has proved challenging because T cell subsets in the peripheral blood may differ from those in the synovium and Tregs although present, may be dysfunctional [365] Studies have revealed moderate reduction of Treg cells in untreated patients with ERA and TNF inhibition led to improved numbers of Tregs.[367] Also, Treg identification is by the intracellular FOXP3 or the zinc finger protein Helios, which is a major barrier to the isolation of the Treg repertoire for an ex vivo study.[368] A further problem stems from the fact that the inciting antigen for RA is unknown and although the approach to manipulate Tregs in RA is attractive, isolation and expansion of highly specific Treg subsets is currently not feasible.[368]

B cells and Breg cells

B cell depleting approaches with rituximab (an antibody against CD20) now have an established role in RA, particularly for seropositive RA patients who have not had an adequate response to TNF inhibition. Although it has been long known that CD20 is present on the cell surface of all pre-plasma cell stages of B cell differentiation, the exact function of this protein remained unknown.[37] A relatively recent report suggests that CD20 is involved in T cell independent antibody responses.[369] CD20 antibodies are of two types (type 1-

rituximab like and type 2- tositumomab like), and recognise different domains of the surface molecule; not surprisingly, antibody binding to these different domains can have differential effector responses.[368]

Additional approaches targeting CD19 (a cell surface regulator on B cells that establishes signalling thresholds) and CD22 (a lectin like Ig superfamily member that modulates BCR binding strength and CD19 mediated signal transduction, and influences B cell adhesion and survival) and those using indirect B cell targeting by inhibiting BLyS or APRIL are other approaches under investigation.[368] A further potential approach is by targeting Breg cells; data on this interesting population with control function over other immune cells is limited.

A fine tuning to targeting and personalising targeted therapy

Despite an extensive body of literature attempting to predict a response in a particular individual to a particular therapy, no definite established biomarkers have emerged; indeed one view is to focus on achieving remission and to 'forget personalised medicine'.[370] This is probably a simplistic view point as is targeting a single pathway or cytokine in isolation. The intricacies and redundancies of the immune system add to the complexity of identification of a relevant target in real time. Even relatively subtle changes in targeting can have very different effects, as exemplified by monoclonal antibody binding to different loops of the CD20 protein (e.g. the differing effector profiles induced by rituximab and tositumomab, both of which target CD20 but different domains of the receptor) and the differential effects of blocking TNF at the cell membrane or soluble receptor levels.[368] [347] One approach may be a combination of serum and synovial biomarkers in treatment naïve patients and repeating these with both treatment success or failure; this may take into account that personalising therapy may actually be a moving target with activation of an alternative pathway leading to an 'escape' of inflammatory inhibition.

Current gaps in knowledge and specific aims of this work

This review has highlighted several areas where there are gaps in knowledge. These include the relevance of using full joint counts (rather than abbreviated joint counts) in the assessment of disease activity, in particular the ankles and feet and the long term effects of omitting these. There is also limited evidence of any benefit of a treat-to-remission strategy on improving work outcomes in RA. A robust predictor of outcomes is radiographic damage

and as yet there are few established markers that help guide decisions on escalating therapy, and it is probable that analysis of RANKL/ OPG and/ or OSCAR may have a role in this regard. Finally, personalised medicine, in terms of improving the still inadequate therapeutic responses to conventional and biologic DMARDs, is still a distant target for an individual patient and synovial biopsy analyses at the time of diagnosis and therapeutic response and relapse may fulfill this unmet need.

CHAPTER 2. ACTIVE FOOT SYNOVITIS: CRITERIA FOR REMISSION AND DISEASE ACTIVITY UNDERESTIMATE FOOT INVOLVEMENT IN RHEUMATOID ARTHRITIS§

ABSTRACT

Objectives

To determine whether remission criteria underestimate foot involvement in RA in a clinic setting.

Methods

123 RA patients were assessed at baseline and 6 months after commencing a response driven combination DMARD protocol. Remission was assessed using criteria for the 28 joint Disease Activity Score [DAS28(ESR)], Simplified Disease Activity Index (SDAI) and Clinical Disease Activity Index (CDAI) as well as the Boolean based 1981 ACR and the proposed 2011 ACR/EULAR criteria. The prevalence of foot synovitis, and mean foot joint scores in patients meeting each remission criteria were estimated by hurdle (mixed distribution) regression.

Results

At 6 months, the 1981 ACR and 2011 ACR/EULAR which utilise full joint counts (including feet) classified the least number of patients as being in remission (12-14%), with minimal evidence of foot synovitis in these patients. In contrast, foot synovitis was present in a substantial proportion of patients (>20%) meeting DAS28, SDAI, CDAI and the 2011 ACR/ EULAR criteria (clinical or trial) calculated using 28 joint counts. The new 2011 ACR/EULAR remission criteria (Boolean and SDAI≤3.3) behaved differently in terms of detecting residual foot synovitis.

§ This chapter has been published as a paper and has been re-formatted (and the reference numbering updated) to conform to the formatting of the rest of the thesis. The citation for this paper is:

Wechalekar MD, Lester S, Proudman SM, Cleland LG, Whittle SL, Rischmueller M, Hill CL. Active foot synovitis in patients with rheumatoid arthritis: applying clinical criteria for disease activity and remission may result in underestimation of foot joint involvement. Arthritis Rheum 2012;64(5):1316-22.

Although the DAS28, CDAI and SDAI have been validated for assessment of remission in RA, their performance in detecting foot synovitis, which they fail to measure directly, is poor, in contrast to ACR and 2011 ACR/EULAR remission criteria using full joint counts. Thus patients may be at risk of ongoing damage if treatment decisions are made solely on the basis of criteria which omit foot joint assessment.

INTRODUCTION

Rheumatoid arthritis (RA) can lead to rapid development of joint damage and significant long-term disability.[61] Intensive target driven treatment using disease modifying anti rheumatic drugs (DMARDs) can produce substantial improvements in disease activity, and physical function, as well as amelioration of radiographic disease progression. [62,371-373] Therefore, accurate assessment of disease activity and defining remission is critical. Since no single measure can capture all aspects of RA activity, various composite measures have been introduced, starting with the ACR (American College of Rheumatology) Boolean remission criteria in 1981,[64] followed by the Disease Activity Score based on 44 joints (DAS44), later modified to the DAS involving a 28 joint count (DAS28).[65,66] More recently, two further composite disease indices, the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI) have been introduced. [67,68] Despite multiple, potentially additive treatment options, true remission can be difficult to achieve, and cannot be validated by clinical assessment alone. Accordingly, definitions for both remission and minimal disease activity (defined as generally short of, but potentially including complete remission), have been proposed for the DAS28, SDAI and CDAI scores. [374] The most recently proposed remission criteria are the 2011 combined ACR/ EULAR (European League against Rheumatism) criteria. [69,375], which propose two definitions; an index based score (SDAI≤3.3) and a Boolean based definition. In addition, the 2011 ACR/ EULAR criteria propose definitions of remission which are suited to a clinical practice setting in which CRP may not be available. [69]

Foot synovitis is a component of active RA not necessarily measured by other indices. For example, up to 36% of RA patients have been reported to have involvement of foot joints prior to involvement of the hands. [320] Further, MRI scans have detected synovitis and bone oedema in the forefeet of patients with early RA in whom MRI of the finger joints was

normal.[376] Since the DAS28, SDAI and CDAI omit the joints of the feet, it is possible that patients with ongoing foot synovitis may be classified as being in remission, yet be at risk for long-term morbidity as a result of unsuppressed arthritis in the feet. This is compounded by the observation that the CRP and ESR (used in most disease activity indices) may be normal in up to 45% of patients with early arthritis [81] thus potentially further underestimating disease activity.

Previous studies using data from randomised control trials have demonstrated that many patients assessed as being in remission using the above measures have ongoing foot synovitis. [80] This has not been investigated in RA patients in a routine early arthritis clinic setting at defined time points, with comparison of older definitions of remission with the newly proposed 2011 ACR/ EULAR definitions for clinical trials (using the CRP) or for routine clinical practice (excluding the CRP), with or without inclusion of foot joints. This is of particular importance given the fact that achieving remission early in the course of RA has previously been shown to minimise deformity and to translate to maintenance of work capacity. [377,378] The aim of this study was to compare the DAS28, SDAI, CDAI, and the 2011 ACR/ EULAR criteria using 28 joints (which do not assess foot synovitis) with the 1981 ACR and the 2011 ACR/ EULAR Boolean remission criteria using a full joint count, for resolution of foot synovitis in early RA in a clinic setting.

PATIENTS AND METHODS

Patients

One hundred and twenty three consecutive patients with recent-onset RA, recruited from the Early Arthritis Clinic (EAC) at the Royal Adelaide Hospital, Adelaide, Australia were included in the study. Patients were included if they were over 18 years of age, had given written informed consent and met the 1987 revised ACR RA classification criteria i.e. symptoms of polyarthritis of more than 6 weeks and less than 24 months, SJC ≥3, a TJC ≥6, an ESR >28 mm/h and/ or C-reactive protein >10 mg/dL. [379] Approval for the study was obtained from the Human Ethics Committees of the Royal Adelaide Hospital and North West Adelaide Health Service.

Study Protocol

Details of the treatment strategy have been published elsewhere. [379] In brief, consecutive patients were recruited for the study, and commenced the EAC response-driven combination DMARD protocol. In this protocol, patients were initially commenced on methotrexate 10 mg orally weekly (progressive increase in the dose of methotrexate (if tolerated) to a maximum of 25 mg/ week), sulfasalazine 500 mg daily orally, increasing over 4 weeks to 2 g daily and hydroxychloroquine 400 mg daily. Predefined adjustments to treatment were made depending on disease activity criteria, with the aim being clinical remission defined as DAS28 score <2.6.

Study evaluations and analyses

Swollen and tender joint counts (SJC, TJC) including those of the feet, visual analogue scores (VAS) for patient and physician-assessed pain, CRP (mg/dL), ESR (Westergren method) and fatigue were recorded for each patient at baseline and 6 months after commencing the combination DMARD protocol.

Remission was assessed 6 months after commencement of DMARD therapy. For the disease activity scores, remission was defined as DAS28-ESR <2.6, SDAI ≤3.3, CDAI ≤10. [65,66,68,70] Remission was also assessed using the original ACR 1981 [64] remission criteria (fulfilment of 5 out of 6 variables: <15 minutes early morning stiffness, no fatigue, no joint pain by history, no joint tenderness, no swelling, and ESR <30 mm/h for women and <20mm/h for men) and by the recently proposed ACR/ EULAR Boolean criteria [69] [remission defined as scores on TJC, SJC, CRP (in mg/dl) and patient global assessment (PtGA, 0-10 scale) all ≤1]. The latter was calculated using the full joint count (including foot and ankle joints) as is recommended and the 28 joint count (excluding foot and ankle joints), which is permitted but not mandated. Remission was also calculated using 28 [remission defined as scores on TJC, SJC and PtGA all ≤1] or full joint counts [remission defined as scores on TJC, SJC, and PtGA all ≤1] by the proposed 2011 ACR/ EULAR remission criteria for use in a clinic setting (in which the CRP is excluded). [69]

Statistical analyses

Agreement between various remission criteria was measured by the prevalence adjusted, bias adjusted kappa (PABAK) statistic, estimated in WinPepi v11.1.[380]

The influence of the respective remission criteria on foot synovitis (SJC+TJC) was analysed by hurdle regression.[381] This analysis utilises a mixture of two distributions, a binomial for modelling the proportion of patients with foot synovitis and a left truncated Poisson for modelling the mean joint counts in those patients with foot synovitis, and resulted in a substantially better fit to the observed data than any single distribution model. All remission criteria resulted in a statistically significant reduction in foot synovitis, indicating some specificity of the criteria for foot synovitis, however, for simplicity, only the fitted values in patients meeting the respective criteria have been reported. Hurdle model were estimated using R v2.12.1 and the pscl package. [382,383]

RESULTS

Baseline patient characteristics

The baseline characteristics of patients are shown in **Table 1**. The presence of high disease activity was evident using all disease activity measures.

Table 1: Baseline characteristics of patients. *

Parameter	Baseline Value
Age at onset (median, range)	58.5 (30-80)
Female	94/122 (77%)
Rheumatoid Factor positive	63/121 (52%)
Anti-CCP positive	68/122 (56%)
Erosive disease	40/116 (34%)
DAS28-ESR (Mean, Range)	5.8 (3.0,8.3)
CDAI (Median, Range)	30.9 (6.8, 69.8)
SDAI (Median, Range)	33.6 (9.4, 71.4)

* Anti-CCP, anti-cyclic citrullinated peptide; DAS28-ESR, 28-joint count Disease Activity Score; CDAI, Clinical Disease Activity Index; SDAI, Simplified Disease Activity Index

At 6 months, there were improvements in the DAS28, SDAI and CDAI and the mean foot TJC and SJC ($p<0.004$, data not shown).

Rates of remission and kappa agreements between the different criteria are shown in **Table 2**.

Table 2. Agreement between the respective remission criteria at six months following commencement of disease modifying antirheumatic drug therapy. *

		Prevalence-adjusted, bias-adjusted kappa							
					2011 ACR/ EULAR criteria (28-joint count)		2011 ACR/ EULAR criteria (full joint count)		
Remission Criteria	Proportion meeting criteria	DAS28-ESR <2.6	CDAI ≤2.8	SDAI ≤3.3	Clinical practice	Clinical trial	Clinical practice	Clinical trial	1981 ACR criteria
DAS28 <2.6	0.29	*							
CDAI ≤2.8 (28-joint count)†	0.18	0.67	*						
SDAI≤3.3 (28-joint count)‡	0.19	0.69	0.95	*					
2011 ACR/ EULAR (28-joint count)									
Clinical practice §	0.13	0.61	0.87	0.85	*				
Clinical trial ¶	0.13	0.61	0.84	0.82	0.97	*			
2011 ACR/ EULAR (full joint count)							*		
Clinical practice §	0.11	0.58	0.84	0.82	0.93	0.90			
Clinical trial ¶	0.10	0.58	0.80	0.79	0.90	0.93	0.97	*	
ACR 1981 (full joint count)	0.08	0.53	0.76	0.71	0.76	0.72	0.82	0.79	*

* DAS28-ESR, 28-joint count Disease Activity Score; CDAI, Clinical Disease Activity Index; SDAI, Simplified Disease Activity Index

† Defined as a composite score criterion in the 2011 American College of Rheumatology/ European League Against Rheumatism (ACR/ EULAR) criteria for remission in a clinical practice setting

‡ Defined as a composite score criterion in the 2011 ACR/ EULAR criteria for remission in rheumatoid arthritis clinical trials [equivalent to the CDAI, with inclusion of the C-reactive protein (CRP) level]

§ Defined as Boolean-based criteria for remission in a clinical practice setting

¶ Defined as Boolean-based criteria for remission in rheumatoid arthritis clinical trials (equivalent to the clinical practice criteria, with inclusion of the CRP level)

The two Boolean based remission criteria utilizing full joint counts (the 1981 ACR and the 2011 ACR/EULAR criteria using full joint count) identified the least number of patients in remission (8% vs 10% respectively) and there was moderately good agreement between these two criteria (kappa = 0.82). The 2011 ACR/EULAR Boolean criteria permit the use of the 28 joint count, which resulted in an increase in the proportion of patients classified as being in remission (13% vs 10%). The 2011 ACR/EULAR remission criteria [69] also include an index

based definition of remission (SDAI≤3.3) that identified a higher proportion of patients in remission (19%) than the Boolean criteria (kappa 0.82). In general, the different index based criteria identified similar proportions of patients meeting the criteria for both remission, but with varying degrees of agreement. The CDAI and SDAI score calculations are closely related, differing by the inclusion of the CRP in the SDAI index and as expected, demonstrate a very high level of agreement (kappa >0.95); the poorest agreements were consistently observed with the DAS28.

The relationship between foot joint involvement and remission criteria

Foot synovitis in patients meeting the various remission criteria was assessed by the both the prevalence of foot synovitis (defined as a combined tender and swollen joint count ≥1), and the mean combined tender and swollen joint counts in those patients with foot synovitis. The results are shown in **Table 3**.

Table 3. Presence of foot synovitis and foot joint counts in patients meeting the respective remission criteria at six months following commencement of therapy. *

Remission Criteria Met	% patients with foot synovitis	Mean foot joint counts (SJC+TCJ) in patients with foot synovitis
DAS28 <2.6	43	2.3
CDAI ≤2.8 (clinical practice)	28	3.6
SDAI ≤3.3 (clinical trial)	27	3.6
2011 ACR/ EULAR criteria (28-joint count)		
Clinical practice	19	3.6
Clinical trial	22	3.6
2011 ACR/ EULAR criteria (full joint count)		
Clinical practice	0	0
Clinical trial	0	0
ACR/ EULAR trial	0	0
ACR 1981	20	0.9

* Fitted values were estimated from the hurdle (zero inflated, Poisson mixture) model as described in Patients and Methods. SJC, swollen joint count; TJC, tender joint count; DAS28-ESR, 28-joint count Disease Activity Score; CDAI, Clinical Disease Activity Index, SDAI, Simplified Disease Activity Index; ACR/ EULAR, American College of Rheumatology/ European League Against Rheumatism

There was minimal evidence of foot synovitis in patients meeting the Boolean 2011 ACR/EULAR criteria which utilised full joint counts (including the feet). Even though 20% of patients had evidence of foot synovitis when using the 1981 ACR criteria, the average joint count in these patients was very low (0.9). In contrast, there was evidence of substantial foot synovitis in patients meeting the various remission criteria which utilized the 28 joint count (which excludes the feet). However, there were no pronounced differences between the performances of these three index based scores in relation to foot synovitis. Therefore approximately one third of patients classified as being in remission by criteria using the 28 joint count had evidence of foot synovitis with an average of 2-3 tender/ swollen foot joints. Furthermore, similar numbers of patients had evidence of foot synovitis using the 2011 ACR/ EULAR clinical (i.e. excluding the CRP) and trial (i.e. including the CRP) criteria if only 28 joint counts were used (19% vs 22%), with an excellent correlation between these two criteria (kappa >0.90).

These results confirm that measures utilising 28 joint counts do not adequately capture persistence of foot synovitis (with 20-30% patients classified as being in remission having active synovitis in an average of in 4 foot joints) and that this is also true with respect to the new 2011 ACR/EULAR criteria, with or without using the CRP; these criteria -when full joint counts are used- identify persistence of foot synovitis equally well, also regardless of whether the CRP in the calculation. Clearly then, the 2011 ACR/ EULAR Boolean criteria using full joints counts are superior to the other criteria with regards to identifying patients in 'true' remission (including feet).

DISCUSSION

The goal of medical treatment in RA has become the achievement of remission. Given that remission itself is difficult to define and substantiate clinically, it is best regarded as a state at the very end of a continuum of diminishing disease activity. Various criteria have been proposed and subsequently validated for measuring remission, including the ACR, DAS, DAS28, SDAI and CDAI. [64-68] These criteria however, vary in their stringency regarding the definition of remission with the DAS28 being the least and the ACR criteria being the most stringent. [77,88,89] The stringency of the definition is of particular relevance not only in 'capturing' synovitis of foot joints, but because in most patients, tighter control leads to better functional and radiographic outcomes,[378,384] notwithstanding the fact that joint damage

progresses in some patients despite good disease control and never progresses to erosive disease in others with continuing joint inflammation. The latter may be especially true in patients receiving aggressive treatment, particularly with a combination of TNF inhibitors and methotrexate.[385] The variable progression probably also reflects the fact that while disease activity is a continuum, these criteria are statistical constructs designed to summarise information on a patient cohort as a whole, and may not necessarily be predictive in a given individual.

The DAS (or DAS44 which includes the feet) was proposed by EULAR in the early 1990s, and is a composite, single-point, absolute measure of disease activity.[79] Subsequently, the DAS28, a new and less time costly score was proposed.[65,66] The DAS28 has been criticized for its omission of the ankles and feet,[80] and defining remission (DAS28 <2.6) on the basis of the DAS28 has engendered controversy with regards to bounding values,[83] for which a more recent criterion has been proposed.[70] The acute phase reactants (CRP / ESR) weigh heavily in the DAS28 calculation, which may erroneously lower the DAS28 score in the face of objective evidence of ongoing joint activity,[84] especially as a significant proportion of patients with RA can have normal ESR and CRP at presentation,[81] including some with radiographic evidence of progressive erosive disease.[386]

The complexity and requirement of computational tools for calculation of the DAS28 was the driving process behind development of the SDAI, a simple numerical summation of values for disease activity parameters (28 TJC, 28 SJC, CRP and both evaluator's (EGA) and patient's global disease activity scores (PGA) on a 10 cm VAS). [68] Subsequently the CDAI was developed as an abbreviated form of the SDAI, excluding CRP from the formula in order to enable the physician to make immediate therapeutic decisions regarding intensification of therapy when laboratory data were not available, because the acute phase reactants correlate with each of the other variables and may not add importantly to a composite score. [68]

The DAS28, SDAI and CDAI all take into account only 28 joints and are conspicuous in their omission of joints of the ankle and foot joints. Consequently, it follows that arthritis in these joints may be missed along with its potential for long term joint damage. It has previously been shown that pain continues to be a problem in a substantial proportion of patients in DAS28 remission[387] and a study using observations from a randomised clinical trial[80] compared DAS28 remission with DAS remission and concluded that the DAS remission is

more stringent than DAS28 remission with the omission of feet joints predominantly responsible for the discrepancy between these measures. Another study which evaluated forefoot disease activity in RA patients found on average that 40% had at least one MTP involved despite being in remission according to the DAS28.[388] A third study that looked at the ankles and feet as a 'block' of 4 joints did not find substantial differences with regards to disease activity indices whether or not ankles or feet were included. This study differed from ours in that it did not necessarily include patients with early arthritis (mean disease duration was 8 years); 34% of patients in this study, despite being in DAS28 remission had evidence of foot synovitis.[389]

Our study addressed this question in a routine, but orderly, early arthritis clinic setting rather than in a clinical trial or in patients with predominantly established RA as has been undertaken previously,[80,389] and has confirmed that disease activity indices utilising 28 joint counts do not adequately capture resolution of foot synovitis. The new 2011 ACR/ EULAR criteria recognise that residual disease activity can be present in the feet of patients deemed to be in remission and recommend but do not require, inclusion of ankles and forefeet in assessment of remission.[69,375] We observed that the Boolean 2011 ACR/EULAR remission criteria using the full joint count adequately captured the resolution of foot synovitis, whereas the composite index based SDAI $\leq$3.3 remission criteria, or the Boolean criteria, both utilising the 28 joint counts, did not. The 2011 ACR/ EULAR remission criteria in their candidate definitions of remission proposed other definitions more useful in the clinic setting in which inflammatory markers may not always be available, and we have assessed disease activity using these indices with or without foot joints. These included a Boolean measure comprising TJC, SJC and PtGA that provided statistically similar results to those obtained with the same measures that included CRP and those with CDAI. [69] A very recent study [390] examining the various proposed remission criteria that excluded foot joints, found that when applied to individual patients, these remission criteria do not necessarily identify the same patient. This has important implications for their use in the individual patient in whom alterations to DMARD therapy need to be made as opposed to the trial setting where the results of the overall group are more relevant.

In summary, although the DAS28, CDAI and SDAI have been validated for assessment of minimal disease activity and remission in RA, their performance in predicting foot synovitis, which they fail to measure directly, is poor in a clinic setting. This failure to assess foot

involvement may lead to less intensive treatment than would otherwise be the case, subjecting patients to the risk of unsuppressed foot joint inflammation and potentially leading to pain, disability and structural failure. This has implications when treatment decisions are being made solely on the basis of disease activity scores which omit assessment of joints of the feet.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Wechalekar had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Dr Wechalekar collected data on foot synovitis and drafted the first version of the manuscript.

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CHAPTER 3. ACTIVE FOOT SYNOVITIS IN PATIENTS IN APPARENT REMISSION IS ASSOCIATED WITH UNSTABLE REMISSION STATUS, RADIOGRAPHIC PROGRESSION AND WORSE FUNCTIONAL OUTCOMES

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory disorder that can lead to progressive and irreversible joint damage. Since intensive target-driven treatment with disease modifying anti-rheumatic drugs (DMARDs) can produce substantial improvements in disease activity (DA), physical function and prevent radiographic progression, using the appropriate target (usually a clinic-based composite DA measure) could be critical to long-term outcome, regardless of the treatment strategy employed.

Various composite DA and remission measures have been validated and in use over the last few years. These include criteria that require a full joint count (e.g. the American College of Rheumatology (ACR) Boolean-based criteria),[64] and those that employ abbreviated joint counts that omit assessment of the ankles and feet. The latter criteria include the DA score involving a 28-joint count (DAS28),[66] the Simplified Disease Activity Index (SDAI) and the Clinical Disease Activity Index (CDAI)[68]. Of these, remission by SDAI has been adopted as an index based remission criteria in the recently proposed 2011 ACR/ European League Against Rheumatism (EULAR) definition of remission.[69]

We have previously shown in a cross-sectional study[82], that agreement between various remission criteria is variable, with the best agreement between the CDAI and SDAI (as expected as these two indices differ only by the inclusion of the CRP in the SDAI), and the poorest with the DAS28. In addition, our study revealed that DA measures that omit foot joints perform poorly in capturing foot synovitis, thus placing patients at risk of ongoing damage if treatment decisions are made solely on the basis of these DA measures.[82] This is particularly important as the feet were the initial site of involvement in early RA in a third or more of patients in a study by van der Leeden *et al*. and ~90% reported painful ankles or feet at some point during the course of their disease.[391] Indeed, pain and swelling of ≥ 1 metatarsophalangeal (MTP) joint was present in 40-50% of patients after 2 years.[391] Among those in remission as calculated by the DAS28, 40% had at least one involved MTP joint (pain and/or swelling).[388] From the standpoint of clinical and long-term outcomes,

radiographic progression is a robust longitudinal outcome measure.[392] This may be particularly relevant for radiographic foot damage, given that foot joints exhibit erosive changes and joint space narrowing (JSN) more frequently at baseline and over time in comparison to hand joints in early RA.[393] With respect to function and health-related quality of life, the multidimensional consequences of RA-related foot synovitis are studied best using patient-reported outcome measures (PROMs). Of these, the health assessment questionnaire (HAQ) and the Medical Outcome Study Short-Form 36 (SF-36) are probably the PROMs that are the best validated in RA. In addition, the SF-36 bodily pain and vitality subscales are the most responsive to improvement.[394,395]

There are limited data regarding the radiographic and functional outcomes of foot synovitis. In the longitudinal study reported by van der Leeden *et al.,*[391] 19% of patients had erosion scores ≥ 1 in the forefeet at baseline. This increased to ~60% after 8 years and the mean score increased from 1.3 to 7.9. This study evaluated walking disability using the HAQ, and reported 57% and 40% walking disability at baseline and one year respectively. Another study[396] found that the DAS28 underestimated DA in those who had progression of radiographic damage in the feet (25% of the study population).

The objectives of this study were to investigate the long term radiographic and functional outcomes of foot synovitis at three years in intensively, predominantly conventional DMARD treated patients, with the treatment strategy aiming for remission. Our specific aims were (1) to investigate dynamic correlations (DCs) between DA measures and foot synovitis, (2) analyse the radiographic progression in patients with foot synovitis, and (3), to assess functional outcomes of foot synovitis over time.

METHODS

Participants

Our sample comprised consecutive adult (>18 years) patients with early (<12 months) RA presenting to the Early Arthritis Clinic (EAC) at the Royal Adelaide Hospital (RAH) between 2000-2014. . Patients were included in this study if they were DMARD-naïve (use of anti-malarials for < 1 month prior to inclusion was permitted) and had RA according to the 1987 revised ACR[12] and/or the 2010 ACR/EULAR criteria.[397] Exclusion criteria were antinuclear antibody titre ≥1:320, evidence of hepatitis B, hepatitis C or HIV infection, recent

seroconversion to parvovirus, Ross River virus, Barmah Forest or rubella viruses, known sensitivity to methotrexate (MTX), sulphasalazine or hydroxychloroquine and systemic disease likely to increase the risk of toxicity to one of these drugs. The study was approved by the RAH Human Research Ethics Committee.

Study Protocol

Details of the EAC cohort, treatment strategy, and results have been published elsewhere.[379,398] Briefly, all patients were randomised to receive high dose or low dose fish oil in addition to treatment with initial triple DMARD therapy (methotrexate 10mg weekly, sulfasalazine 500mg twice daily increased to 1g twice daily over 3 weeks and hydroxychloroquine 200mg twice daily). Treatment was escalated to achieve DAS28(ESR) remission by initially increasing methotrexate to a maximum of 25mg weekly followed by addition of leflunomide, other DMARDs or a biologic DMARD. If clinically deemed necessary, patients could have parenteral glucocorticoids (typically 120 mg IM depot methyl prednisolone). The use of oral glucocorticoids and NSAIDs was actively discouraged and if used at study entry, they were tapered and ceased where possible.

Study evaluations and analyses

The primary outcome of the EAC study was failure of triple DMARD therapy to achieve remission as defined by the DAS28(ESR) <2.6; failure was defined as requirement to progress to addition of leflunomide. Patients were reviewed every 3-6 weeks. During each visit, joint counts (JC) were assessed as tender (53 joints and 28 joints; TJC) and swollen (44 joints and 28 joints; SJC) joint counts; a 100 mm visual analogue scale (VAS) was used to assess patient and physician global activity, pain and fatigue. Quality of life measures assessed included the modified health assessment questionnaire (mHAQ; every visit) and SF-36 (yearly).

Composite measures of DA assessed, in addition to the DAS28(ESR), were the DAS28(CRP), CDAI and SDAI. For these measures, remission was defined as a DAS28-CRP score of <2.6, CDAI score of ≤2.8 or SDAI score of ≤3.3.[67,68,399] Patients had yearly radiographic assessments, which were scored using the Sharp modified van der Heijde (SvH) method [139] by two independent observers (SP, MW). Serial hand and feet radiographs were blinded to identity of participants but not to chronological order.

Statistical analysis

Statistical analysis was done using the R program for statistics.[382] Dynamic (within patient) correlations between DA scores and foot TJC and SJC scores over were estimated in patients with at least three treatment observations over three years' of follow up (n=217) using the R library dynCorr,[400] with 95% confidence intervals (CI) estimated by bootstrapping. The average prevalence (over all treatment visits) of (1) remission according to different DA criteria and (2) foot synovitis (defined as any tender or swollen joints) in patients in remission, was estimated using marginal binomial generalised estimating equations models in patients with at least one treatment visit (n = 266). Transitions between disease remission and non-remission states over time were analysed using a multi-state Markov model, using the R library msm [401] in patients with at least two treatment visits (n=263), and results are reported as the average length of stay ("sojourn time") in either a remission or non-remission state for the different DA remission criteria. A second analysis included foot synovitis as a covariate and results are reported as hazard ratios for the effect of foot synovitis on the transition intensity for each transition direction.

Radiographic scores (n=238) over time were analysed using a random intercept, marginal exponential growth model to calculate the average annual "growth" rate in scores. This was estimated using a negative binomial mixed regression model (log-link), rather than a Poisson regression model, because of overdispersion in the radiographic scores. This analysis was performed using the R library glmmADMB.[402]

For SF-36 data (n=255), each SF-36 domain scale (0-100) was transformed to a norm-based scale (NBS), with a mean of 50 (SD 10), using age and gender matched South Australian population data,[403] to enable direct comparison between results of each domain. Mixed effects linear regression model was applied to analyse associations between disease activity and SF-36 scores, using the R library nlme.[404] To analyse the association of foot synovitis and SF-36 scores with foot synovitis as an additional predictor variable, foot joint scores were transformed with a square root transformation prior to analysis, because of the variable and skewed nature of foot joint counts.

Baseline patient characteristics

The baseline characteristics of patients are presented in **Table 1**. The mean age was 55, 71% were women and 48% were current or past smokers. In this cohort, the prevalence of rheumatoid factor (RF), anti-CCP (cyclic citrullinated peptide) and the shared epitope was 60%, 51% and 59% respectively. Mean baseline DAS28(ESR), SDAI and CDAI were consistent with high disease activity; mean mHAQ was 0.756 and mean duration of symptoms prior to diagnosis was 23 weeks. Baseline total (median) SvH score was 4; 27% of patients had erosive disease at baseline.

DCs between DA scores and foot joint scores

Dynamic correlations (DCs) were obtained a minimum of 3 observations per patient, during treatment; observations were from six months to three years. The DCs **(Table 2)** are those within individuals, over time. We found that all DA scores showed a weak to moderate positive correlation with foot swollen joint counts (SJC) and tender joint counts (TJC) and the strength of these correlations improved when both SJC and TJC were considered. Correlations were comparable for DAS28(ESR)/DAS28(CRP) and SDAI/ CDAI. Despite the statistical significance of these correlations, the DA scores 'captured' less than 50% of the variation in foot SJC/TJC counts, indicating that assessment of disease activity using these criteria is likely to be insufficient for detecting disease flares in the feet.

SDAI and CDAI are more stringent remission criteria, yet a significant proportion of patients in remission may still have foot synovitis

The percentage of patients achieving SDAI and CDAI remission in this cohort was less than that achieved with DAS28 criteria. However, even with these newer criteria, 24% in SDAI remission and 25% of patients in CDAI remission had ongoing foot synovitis **(Table 3)**.

Table 1. Baseline demographic and clinical data (n=266)

	n (%), mean (SD) or median (IQR)	
Age, years, mean (SD)	55	(14)
Females (%)	190	(71)
Ever smoked (%)	128	(48)
BMI, mean (SD)	27.9	(6.2)
RF positive (%)	161	(60)
Anti-CCP positive (%)	136	(51)
Shared Epitope positive (%)	157	(59)
Symptoms prior to diagnosis, weeks, mean (SD)	23	(32)
ESR, median (IQR)	29	(31)
CRP, median (IQR)	10	(24)
SJC, median (IQR)		
Total	10	(11)
SJC28	7	(8)
Ankles & feet	2	(5)
TJC, median (IQR)	17	(18)
Total	17	(18)
TJC28	10	(11)
Ankles & feet	6	(9)
Proportion of patients with foot synovitis (TJC and/or SJC) (%)	219	(82)
DAS28(ESR), mean (SD)	5.43	(1.27)
DAS28(CRP), mean (SD)	4.99	(1.18)
SDAI, mean (SD)	31.70	(14.50)
CDAI, mean (SD)	29.70	(13.80)
mHAQ, mean (SD)	0.756	(0.549)
SvH radiographic score, median (IQR)		
Total score	4	(8)
Proportion of patients with erosive disease (%)	64	(27%)
Erosion scores in patients with erosive disease (IQR)	1.0	(3.0)

BMI, body mass index; RF, rheumatoid factor; CCP, cyclic citrullinated peptide; CRP, C-reactive protein; SJC, swollen joint count; TJC, tender joint count; SJC/TJC 28, SJC/TJC using 28 joint count; DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index; mHAQ, modified health assessment questionnaire; SvH, van der Heijde modified Sharp score; JSN, joint space narrowing; IQR, inter quartile range.

Table 2. Dynamic correlations between disease activity scores and foot tender (TJC) and swollen joint counts (SJC)

DA	Foot.SJC*	Foot.TJC*	Foot SJC + TJC*
DAS28(CRP)	0.24 (0.10, 0.37)	0.30 (0.16, 0.40)	0.35 (0.21, 0.43)
DAS28(ESR)	0.22 (0.08, 0.35)	0.30 (0.16, 0.42)	0.34 (0.20, 0.44)
SDAI	0.32 (0.12, 0.46)	0.40 (0.24, 0.51)	0.46 (0.28. 0.55)
CDAI	0.31 (0.11, 0.46)	0.40 (0.24, 0.51)	0.46 (0.28, 0.55)

* The numbers refer to the dynamic correlation within patients over time (r) (95% CI); DA, disease activity; SJC, swollen joint count; TJC, tender joint count; DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index

Table 3. Foot synovitis in patients in remission

	DAS28 (CRP)*	DAS28 (ESR)*	SDAI*	CDAI*
Patients in remission†	0.47 (0.11, 0.51)	0.43 (0.10, 0.47)	0.28 (0.05, 0.32)	0.27 (0.05, 0.31)
Patients in remission with foot synovitis	0.35 (0.29, 0.41)	0.36 (0.30, 0.42)	0.24 (0.18, 0.30)	0.25 (0.19, 0.32)

* The numbers refer to the proportion of patients (95% CI). † These include patients in remission regardless of presence or absence of foot synovitis. DA, disease activity; SJC, swollen joint count; TJC, tender joint count; DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index.

In early disease, SDAI/CDAI remission is less sustained than DAS28 based remission and the presence of foot synovitis predicts relapse among those in DAS28, CDAI and SDAI remission

As might be expected, we found that in the first 3 years, RA disease activity fluctuates and many patients transition between remission and non-remission, with the transitioning occurring in both directions. When we estimated the average length of stay in any one state (the "sojourn time"), we found that the sojourn time in remission for SDAI/CDAI is substantially shorter than that for DAS28 based remission **(Table 4)**.

Table 4. Average sojourn time (years) for remission states by DA score

State	DAS28 (CRP)*	DAS28 (ESR)*	SDAI*	CDAI*
Non Remission	1.6 (1.3, 2.0)	1.7 (1.4, 2.1)	1.9 (1.4, 2.6)	2.1 (1.6, 2.7)
Remission	2.0 (1.5, 2.7)	2.0 (1.5, 2.7)	1.0 (0.6, 1.4)	1.0 (0.7, 1.5)

* The numbers refer to the sojourn time in years (95% CI). DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index

When we included foot synovitis as a covariate to determine if presence of foot synovitis affected the probability of transitioning from remission to non-remission, we found that foot synovitis significantly influences the transition intensities (as assessed by the likelihood ratio p-value), and patients in DAS28(ESR), SDAI and CDAI remission with foot synovitis were approximately twice as likely to relapse compared to patients in remission without foot synovitis **(Table 5)**.

Table 5. Hazard ratios (95% CI) for the effect of foot synovitis on transition intensities from remission to non-remission and vice versa

Transition	DAS28(CRP)*	DAS28(ESR)*	SDAI*	CDAI*
Non Remission to Remission	0.68 (0.42, 1.10)	0.70 (0.45, 1.10)	0.70 (0.40, 1.24)	0.68 (0.39, 1.17)
Remission to Non-Remission	1.47 (0.84, 2.57)	1.81 (1.03, 3.17)	2.06 (1.00, 4.26)	2.08 (1.03, 4.18)
Likelihood Ratio test p value	0.015	0.002	0.0004	0.0002

*The numbers refer to hazard ratios (95%CI). DA- disease activity; SJC, swollen joint count; TJC, tender joint count; DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index

Radiographic progression

We analysed progression of both JSN and erosion scores over time. Because of the log-link employed in the regression model, the estimated average annual growth (i.e. increase) in scores represents the ratio of scores between successive yearly intervals, as opposed to the arithmetic difference in scores. The score "growth rate" is therefore strongly influenced by factors affecting the baseline score, and hence it was important to model these appropriately in all analyses.

Age was the most important predictor of baseline JSN, with higher scores occurring among older patients ($p < 0.001$). There were no clear relationships between JSN (either baseline or change over time) and other potential predictor variables (anti-CCP, smoking, duration of disease prior to treatment, body mass index (BMI), gender and disease activity) after adjusting for age.

Both baseline age and anti-CCP status were highly significant predictors of baseline erosion scores, but did not affect the progression ("growth rate") of erosion scores. There were no significant associations with other baseline variables including gender, smoking status, duration of symptoms prior to treatment, BMI and baseline disease activity scores. The coefficients of the final regression model are summarised in **Table 6**.

Table 6. Regression Coefficients for analysis of effect of baseline age and anti-CCP status for progression of erosion scores

Coefficient	Estimate	SE[#]	p-value
(Intercept)	-2.300	0.278	<0.001
Age.c *	0.042	0.013	0.0012
anti-CCP status[**]	0.551	0.194	0.0044
Time [***]	0.343	0.034	<0.001

[#]SE, Standard Error. All regression coefficients are in (natural) log format and can be interpreted by exponentiation. [*]The Age.c predictor variable is the baseline age, centred around the mean age of 55 years. **For dichotomous anti-CCP status, contrast sum coding (i.e. (-1,1) instead of dummy (0,1) coding) was used. Therefore the intercept estimates the (log) baseline score at the mean of both age and anti-CCP. [***] The Time coefficient represents the log of the average annual growth rate of erosion scores.

There was a significant confounding between age and anti-CCP status. Patients with anti-CCP were, on average, younger, and the importance of anti-CCP status as a baseline predictor of erosion scores only became apparent after first adjusting for age (i.e. effectively measuring the effect of anti-CCP in patients of the same age). From the regression coefficients in Table 6, we can estimate that the ratio of erosion scores, at any given time point, for a patient who was 65 years old, relative to a patient who was 55 years old as exp(10*0.042)= 1.53 (95% CI 1.18, 1.98), i.e. about 50% higher. Also, the estimated ratio of erosion scores, at any given time point, for an anti-CCP positive patient as compared to an anti-CCP negative patient was 3.01 [95% CI 1.41, 6.84; calculated as exp(2*0.551)] i.e. a 3 fold difference. For patients with the same baseline age and anti-CCP status, the ratio of erosion scores at three years relative to their baseline score was estimated as 2.80 [95% CI 2.30, 3.41; calculated as exp(3*0.343)] i.e. nearly a 3 fold increase over three years.

Sustained DA remission markedly reduces the growth rate of erosion scores

This analysis was performed by calculating a variable ("DA remission.ave"; **Table 7**) for the proportion of times a patient was in DA remission over the 4 treatment time points (6 months, 1 year, 2 years and 3 years). A Time:DA remission.ave interaction variable was added to the previous model. This interaction term measured whether DA remission was associated with

the average annual growth rate. The regression estimates for the 4 different DA scores are presented in **Table 7**.

As for the previous analyses, age was centred at the mean of 55 years and the "contrast sum" contrasts (as described in the foot note to Table 6) was used to dichotomise anti-CCP status. In this model, the "Time" coefficient reflects the (log) growth rate in erosion scores for patients who were never in remission; the "Time:DA.remission.ave" coefficient reflects the difference in the (log) growth rate for patients with varying success in achieving remission compared to those who never achieve remission. We found the coefficient here to be negative, and highly significant for each DA score, suggesting that the more remission is sustained, the more pronounced the benefit of remission in ameliorating the increase in erosion scores. This is particularly relevant, as the transition state analysis of the sojourn time presented earlier has shown that remission is more unstable in the presence of foot synovitis.

We wished to identify if the duration of DA remission would predict those patients with an increased ratio of three year to baseline erosion scores (among individuals of similar age and anti-CCP status). Remission status was given a semi-quantitative score (never in remission, 50% remission, always in remission; **Table 8)** and results suggest that erosion scores may still progress (by nearly two-fold) over 3 years in patients who have sustained DAS28(CRP) or DAS28(ESR) remission, in contrast to no statistically significant progression over 3 years in those in sustained SDAI and CDAI remission. Although some of the differences in p-values between DAS28 and SDAI or CDAI remission may reflect statistical power (i.e. since SDAI/CDAI remission is more stringent, so it identifies fewer patients in remission), we also found that the estimated growth rate of erosion scores was lower for those in SDAI/CDAI remission.

Table 7. Regression estimates for DA measures and growth rate of erosion scores

	DAS28(CRP)			DAS2(ESR)			SDAI			CDAI		
	Estimate	SE	p	Estimate	SE	P	Estimate	SE	p	Estimate	SE	P
(Intercept)	-2.205	0.267	<0.001	-2.206	0.268	<0.001	-2.216	0.269	<0.001	-2.218	0.269	<0.001
Age.c	0.044	0.013	0.001	0.043	0.013	0.001	0.044	0.013	0.001	0.044	0.013	0.001
Anti-CCP positive	0.538	0.192	0.005	0.543	0.191	0.005	0.545	0.193	0.005	0.548	0.193	0.004
Time	0.434	0.048	<0.001	0.422	0.047	<0.001	0.413	0.041	<0.001	0.406	0.040	<0.001
Time: DA remission.ave	-0.231	0.085	0.006	-0.210	0.084	0.013	-0.320	0.107	0.003	-0.286	0.105	0.006

SE, Standard Error; DAS28, disease activity score calculated using 28 joint count; SDAI, simplified disease activity index; CDAI, clinical disease activity index

Table 8. Three year to baseline erosion score ratios (results derived from the regression model in Table 7), for varying degrees of DA remission

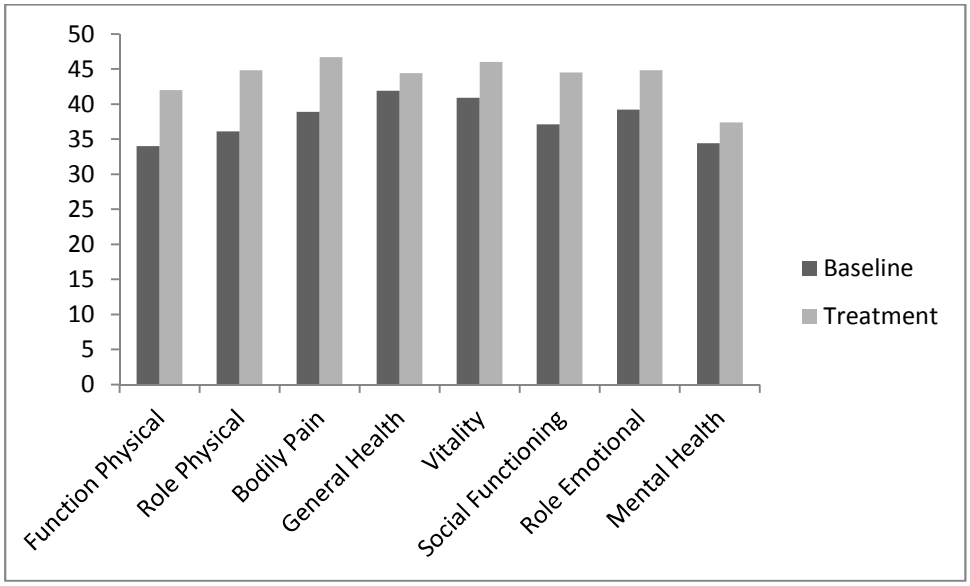
Remission Average	**DAS28(CRP)**		**DAS28(ESR)**		**SDAI**		**CDAI**	
	Ratio (95% CI)	p-value	Ratio (95% CI)	p-value	Ratio (95% CI)	p-value	Ratio (95% CI)	p-value
Never Remission	3.76 (2.78, 4.85)	<0.001	3.54 (2.69, 4.67)	<0.001	3.45 (2.72, 4.38)	<0.001	3.38 (2.67, 4.28)	<0.001
50 % Remission	2.60 (1.78, 3.77)	<0.001	2.59 (1.75, 3.81)	<0.001	2.14 (1.41, 3.23)	<0.001	2.20 (1.49, 3.25)	<0.001
Always Remission	1.84 (1.04, 3.25)	0.037	1.89 (1.05, 3.40)	0.034	1.32 (0.66, 2.65)	0.43	1.43 (0.74, 2.78)	0.29

SDAI, simplified disease activity index; CDAI, clinical disease activity index

DA is strongly associated with scores in each SF-36 domain and foot synovitis is independently associated with Physical Functioning (PF)

Analysis of SF-36 scores (based on the baseline and mean score for each patient during treatment (years 1, 2 and 3)) revealed mean SF-36 NBS at baseline were lower than those after treatment (Figure 1). Even after treatment, SF-36 scores in the EAC cohort were lower than the general South Australian population scores.

Figure 1. SF-36 scores are higher post-treatment for all SF-36 subscales



DA during treatment, as measured by the DAS28(CRP) (as the composite marker used to assess DA and remission in our cohort), was strongly associated with scores in each SF-36 domain, using regression analysis **(Table 9).** Baseline age was centred around 55 years and predictor variables for the SF-36 domain NBS responses were age and DAS28(CRP). The DAS28(CRP) was centred around 2.6, and as a time-varying covariate, included both between patient and within patient effects.

Although scores were normalised to age and gender matched controls, baseline age was still a significant covariate (in contrast to gender) for 7 out of the 8 domains indicating some differences between the RA patients and the population controls in the age-score relationships. Six of the 7 significant age coefficients were positive, possibly indicating a trend for over-correction when normalising RA patients to the population controls. This was most pronounced for Physical Functioning (PF) and General Health (GH). As noted in **Table 9**, the coefficient for DAS28(CRP) was indicative of a substantial effect. The coefficients

remained close to 4 units which means a one unit decrease in the DAS28 score (i.e. from 2.6 to 1.6) is expected to result in an increase in the SF 36 domain NBS of around 4 (and vice versa).

Table 9. Associations of SF-36 domains with disease activity.

SF36 Domain	Regression Coefficients								
	Intercept			Age.c			DAS28.CRP.2.6		
	E	SE	p-value	E	SE	p-value	E	SE	p-value
Physical Functioning	43.7	0.6	< 0.001	0.21	0.04	< 0.001	-4.9	0.4	< 0.001
Role Physical	45.7	0.5	< 0.001	0.08	0.03	0.005	-3.8	0.2	< 0.001
Bodily Pain	48.0	0.4	< 0.001	0.08	0.03	0.002	-4.4	0.3	< 0.001
General Health	45.2	0.5	< 0.001	0.24	0.03	< 0.001	-3.4	0.3	< 0.001
Vitality	47.0	0.5	< 0.001	0.07	0.03	0.023	-3.5	0.3	< 0.001
Social Functioning	46.3	0.6	< 0.001	-0.10	0.04	0.007	-3.0	0.3	< 0.001
Role Emotional	47.0	0.5	< 0.001	0.07	0.03	0.023	-3.5	0.3	< 0.001
Mental Health	38.8	0.6	< 0.001	-0.02	0.04	0.59	-4.0	0.4	< 0.001

E- Estimate, SE- Standard Error

We also analysed the relationship between foot synovitis (foot SJC+ foot TJC) scores and each domain of the SF-36 during treatment, by the inclusion of an additional covariate (the square root of the total synovitis scores). As in the previous analysis, baseline age was centred around 55 years, and DAS28(CRP) was centred around 2.6 and the additional covariate for foot synovitis scores "sqrt.foot.SJC.TJC.c" was centered around $\sqrt{2}$.

We found that the foot joint score is quantitatively related to the SF-36 Physical Functioning (PF), NBS scale, even after adjustment for DAS28(CRP) **(Table 10)**. The regression coefficient was negative, and highly significant for foot joint score, indicating a lower SF-36 PF in the presence of foot synovitis. The other SF-36 domain scores were associated with

foot joint scores only if the DA score was omitted from the model, indicating that they are likely to be secondary associations induced by the correlation between foot joint scores and DA scores.

Table 10. Relationship of the foot joint count to SF-36 Physical Function (PF) (Norm Based Scale- NBS)

Term	Estimate	Standard Error	p-value
(Intercept)	43.4	0.6	< 0.001
Age.c	0.20	0.04	< 0.001
DAS.CRP.2.6[#]	-4.4	0.4	< 0.001
sqrt.Foot.SJC.TJC.c[*]	-0.7	0.3	0.025

[#] DAS28(CRP) centred around 2.6 as the time varying covariate

[*] The predictor variable for combined foot (swollen and tender) joint scores. As these scores were variable and highly skewed, they were transformed prior to analysis with a square root transformation. This transformed variable is "sqrt.Foot.SJC.TJC.c", also a time varying covariate. The intercept is the mean PF NBS for individuals at the mean of the covariates (age =55 years, DAS28CRP= 2.6 and SJC+TJC=2)

DISCUSSION

In this cohort of early RA, our study confirms and extends our previous findings concerning the underestimation of disease activity using DA measures that omit foot joints. When these criteria are used to define remission, a substantial proportion of patients have ongoing foot synovitis, which predicts relapse of standard DA measures, radiographic progression and worse functional outcomes.

The goal of treatment in RA is achievement of remission, which is best regarded as a state at the very end of a continuum of diminishing disease activity. Given that no one single measure is adequate to define remission, composite measures introduced over the years have

attempted to define cut-point values, with varying levels of stringency. Pinals' statement in 1981 that "substantial variation appears to exist in the concept of remission within the group of participating rheumatologists"[64,69] still remains valid. In the current era of biologic DMARDs when remission is a realistic goal, it is critical not only to use stringent remission criteria, but also recognise their limitations, so that we may achieve the best long-term results in individuals. It is in this context that our study assumes particular relevance.

There are limited longitudinal data on the ability of composite measures that use a limited joint count, to "capture" foot synovitis although it is recognised that the SDAI[88] and the original DAS[80] have greater stringency than the DAS28. For example, Landewe *et al*. compared DAS with DAS28 remission using paired observations and found DAS remission to be more stringent than DAS28 remission, with the discrepancy accounted for by the omitted foot joints.[80] Kapral *et al*. [389] compared remission using either a 28 joint count or a 32 joint count (this included the ankles and feet as a 'block' of 4 joints). They found that classification of disease activity remained similar whether 28 or 32-joint counts were used, but that DAS28 remission was frequently recorded in the presence of ankle/foot swelling (34%) and tenderness (31%). When SDAI remission was recorded, persistent ankle/ foot swelling occurred in only 8% of such patient visits. In contrast to our cohort, this last study had patients with longer disease duration at study entry (8 years mean disease duration) and lower baseline disease activity. Our patients had earlier, more active disease and this may account for the increase in persistent foot synovitis among our patients in DA measure remission.

A study from an early RA inception cohort that evaluated MTP synovitis found that up to 40% of patients in DAS28 remission had at least 1 MTP involved.[388] Another study [405] from a different early RA cohort evaluated the new ACR/ EULAR remission criteria [69] using either a 28- or a 38-joint count (the 38 JC included MTPs). Of those who reached remission at 1 year using 28-JC, 26% and 36% had foot synovitis using the Boolean and SDAI definition of remission, respectively. Use of the 38-JC lowered remission rate by only 2-3% by the Boolean definition. There were several limitations to this study. Importantly, mid- and hind-foot joints were not assessed (in contrast to our study in which all foot joints were assessed), the number of patients who were in remission and still had foot synovitis was small and the follow-up with was limited to 1 year. Despite these findings, the authors of this study

emphasised the value of assessing all relevant joints in an individual patient, including the foot joints.

This study identifies that persistence of foot synovitis in common in patients with early RA who have otherwise achieved remission on the basis of standard DA measures. While the newer measures (SDAI and CDAI) classified fewer patients with foot synovitis as being in remission, they still missed up to 25% of those with active foot synovitis. Furthermore, those with more unstable disease (i.e. those who spent less time in remission) were more likely to have active foot synovitis at the time remission was recorded. This has direct consequences. Shorter remission times are associated with an increase in erosion scores (i.e. damage). Foot synovitis is also associated with reduced physical functioning on the SF36, suggesting that it increases morbidity.

We found that shorter remission times are associated with an increase in erosion scores, similar to others who have reported that active disease[406] and disease flares[377] lead to greater radiographic progression. Also, joint damage is preceded by increased disease activity[407] and therefore use of more stringent DA measures may allow earlier titration of targeted therapies in order to reduce radiographic progression. A relatively recent study also reported that 25% of patients mainly have radiographic foot progression, as opposed to 50% who progress to a similar extent in the hands and feet and 25% who progress mainly in the hands. DAS28 underestimates disease activity mainly in those who exhibit radiographic progression in the feet[396] and therefore may underestimate disease activity in 75% of patients. We show, for the first time, the relevance of foot synovitis in this context because our transition state analysis demonstrates that remission is more unstable in the presence of foot synovitis.

Consistent with previous studies, baseline SF-36 scores in RA in our study, for all subscales, were lower than population norms, correlate with disease activity and improve with treatment [394,395,408,409]. We also found that foot synovitis in particular, was related to SF-36 PF scale, even after adjustment for DA, indicating the independent contribution of foot synovitis to the SF-36 PF score. This is particularly relevant as many activities of daily living such as climbing stairs, walking and recreational activity such as sports are captured in the SF-36 score, and may explain why foot synovitis impacts on quality of life.

Our study is one of only a few studies to analyse outcomes of foot synovitis longitudinally in a closely followed treat-to-target inception cohort. To our knowledge, this is also the first study to report the lack of stable remission and worse functional outcomes in the presence of foot synovitis, enhancing and adding to previous knowledge regarding limitations of DA criteria that omit foot joints. The limitations of our study include the lack of specific measures of foot function and pain (such as the Bristol Foot Score). Although this was an open design study, joint examination was conducted by a single metrologist, who was not involved in treatment decisions.

In summary, this study demonstrates that persistent of foot synovitis in common in patients with early RA who have otherwise achieved remission on the basis of standard. DA measures. While the newer measures of DA classified fewer patients with foot synovitis as being in remission, they still missed up to 25% of those with active foot synovitis. Furthermore, those with more unstable disease (i.e. those who spent less time in remission) were more likely to have active foot synovitis at the time remission was recorded. This has direct consequences. Shorter remission times are associated with an increase in erosion scores (i.e.damage) and foot synovitis is also associated with reduced physical functioning on the SF36, suggesting that it increases morbidity. Our findings emphasise the importance of examining the ankle and foot as a part of the routine management of patients with RA. Given the impact of foot synovitis on stability of remission, radiological progression and independent impact on quality of life, decisions should not be made solely on the basis of DA scores that omit foot joints. Presence of foot synovitis despite apparent remission should prompt escalation of therapy to prevent long-term joint damage and improve functional outcomes.

CHAPTER 4. A TREAT-TO-TARGET STRATEGY PRESERVES WORK CAPACITY IN A RHEUMATOID ARTHRITIS INCEPTION COHORT§

ABSTRACT

Objectives

Quantification of work disability in patients with early rheumatoid arthritis receiving conventional DMARDs according to a treat-to-target strategy.

Methods

Patients received combination conventional DMARDs, escalated to achieve DAS28(ESR) remission and completed an annual work and arthritis questionnaire. Random effect mixed modelling was used to assess associations between the average hours worked per week (HWPW), and baseline prognostic factors. HWPW were compared with matched population averages. Cox proportional hazards modelling was employed to evaluate associations between permanent loss of employment and treatment response, disease and demographic factors.

Results

Work data from 299 patients (1562 observations) followed for up to 14 years (range 1-14) were available for analysis. Of those working, mean age was 45 years, 70% were female, and 70% and 68% were seropositive for rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) respectively. Men worked more hours than women; there was a highly significant association between working hours lost and increasing age (0.41 hours, $p=0.003$) and female gender (12.98 hours, $p<0.001$). HWPW were maintained compared to the general population (loss of 0.63 vs. 0.24 HWPW). EULAR good responders at 6 months were more likely to be working at 10 years compared to those with moderate/no response ($p=0.024$); permanent loss of employment and baseline age were strongly associated for anti-CCP positive participants ($p=0.004$).

§ **This chapter is under preparation for submission to Journal of Rheumatology..**

Conclusions

Treat-to-target combination conventional DMARD therapy maintains work capacity, particularly in good responders, comparable to the general population. Improving treatment response in moderate/no responders early in disease may increase work retention.

Significance and Innovations:

- A treat-to-target strategy using conventional combination DMARD therapy in early rheumatoid arthritis preserves work capacity as compared to the general population
- Improving treatment responses in those who do not have a good response to therapy early in disease may be required to increase work retention

INTRODUCTION

Rheumatoid arthritis (RA) has profound societal consequences, including the inability to continue employment. Historical data indicate that up to 30% become work disabled within the first 3 years after diagnosis [169]; this loss increases over time.[164] More than half cease work by a decade after diagnosis, and 90% stop work prior to retirement age.[165] Although work retention has improved over the past two decades,[166,410] this improvement does not extend to those involved in manual occupations and work disability remains unacceptably high. [411]

Work disability in RA can be assessed using biomedical findings (i.e. disease activity and structural damage) and biopsychosocial factors (i.e. a mismatch between functional ability and work demands).[167] Systematic reviews have identified a robust association between work disability and increasing age, functional disability, physically demanding occupations and lower education levels. In contrast, associations with disease activity, structural damage and seropositivity have been inconsistent.[167,169] As disease status ultimately determines work disability,[411] changes to treatment strategies that optimise disease outcomes could translate to improved work outcomes.

Treatment options for those with RA have changed substantially over the past two decades and remission is now an achievable goal. While biologic therapy is an appropriate option for those with resistant disease, intensive target-driven treatment (the “treat-to-target” strategy)

with disease modifying anti rheumatic drugs (DMARDs) has been repeatedly shown to supress disease activity, reduce radiographic progression and to improve physical function.[62,371-373] Clinical outcomes with intensive conventional DMARDs are similar to biologic therapy, particularly in early RA.[412,413]

While clinical outcomes and economic considerations support the use of conventional DMARDs and a treat-to-target strategy, data on work outcomes are limited. The Finnish Rheumatoid Arthritis Combination (FIN-RACo) study found that those who received a treat-to-remission approach with combination therapy (vs. single drug therapy) fared significantly better.[170] The Swedish Pharmacotherapy (Swefot) study also used a treat-to-target strategy and found similar work outcomes between patients treated with conventional or biologic DMARDs, with significant improvement in number of work-days lost over the 21 month follow up period in both arms.[178] These studies suggest that intensive therapy can improve work outcomes, but neither included a treatment arm using a combination of conventional DMARDs without oral corticosteroids or a biologic DMARD.

The objectives of this study were to investigate work disability in patients with early RA treated with a combination of conventional DMARDs, using a treat-to-target strategy without initial oral corticosteroids or biologic DMARD. Our specific aims were to investigate associations with loss of working hours over time, to compare work disability in this population to the general population and to analyse the effects of response to treatment on permanent loss of employment.

PATIENTS AND METHODS

Participants

Our sample comprised consecutive adult (>18 years) patients with early (<12 months) RA enrolled in inception cohorts at the Early Arthritis Clinic at the Royal Adelaide Hospital between 2000-2014 and the Early Synovitis Clinic at the Repatriation General Hospital between 2011-2014, who reported that they were working on at least one occasion.

Inclusion criteria required participants to be DMARD-naïve and have RA according to the 1987 revised American College of Rheumatology (ACR)[12] and/ or the 2010 ACR/ European League Against Rheumatism (EULAR) criteria.[397] The study was approved by the respective Research Ethics Committees.

Study Protocol

Details of the EAC cohort, treatment strategy and results have been published elsewhere [379,398]. Briefly, all patients commenced treatment with initial triple DMARD therapy (methotrexate 10mg weekly, sulfasalazine 1g twice daily and hydroxychloroquine 200mg twice daily). Treatment was escalated to achieve DAS28(ESR) remission by increasing methotrexate to a maximum of 25mg weekly followed by addition of leflunomide, other DMARDs or a biologic DMARD according to a pre-defined algorithm [398]. If clinically deemed necessary, patients received parenteral corticosteroids (typically 120 mg IM depot methylprednisolone); oral corticosteroids and NSAIDs were actively discouraged and if used at study entry, tapered and ceased where possible.

Outcome measures

Clinical

Patients were reviewed in clinic every 3-6 weeks. Joint counts were assessed as tender (53 joints and 28 joints) and swollen (44 joints and 28 joints) joint counts; the composite measure of disease activity used was the DAS28(ESR); EULAR response [414] was used to differentiate good responders from moderate/ no responders at 6 months. A 100 mm visual analogue scale was used to assess patient global activity. The modified health assessment questionnaire (mHAQ) and the helplessness index were completed at each visit.[124] Patients had yearly radiographic assessments, scored using the Sharp modified van der Heijde method [139] by two independent observers (MW, SP), blinded to identity of participants but not to chronological order.

Work disability

Work was assessed annually by a questionnaire, used previously in a cross-sectional study of work participation in established RA.[415] Specifically, the work characteristics ascertained were: paid employment (current or past), hours worked per week (HWPW), level of formal education, occupation, need to change nature of work or hours worked because of RA, and the effect of RA on income. The time to permanent job loss was also ascertained.

Hours worked were compared with age-, gender- and time-matched data from the Australian Bureau of Statistics (ABS)[416] for every month during the study period (April 2001-July 2014). The primary source of ABS labour force survey data is from multi-stage area samples of private dwellings (approximately 26 000 houses, covering 0.32% of the population aged

15 and over), with selected households interviewed monthly for eight months and one-eighth of the sample replaced each month; information is obtained by trained personnel using computer-assisted interviewing or online self-completion.

The current retirement age in Australia is 65. If an Australian resident is unable to temporarily perform their job, they are entitled to sickness allowance, payable between ages 22 and 64 years; employed, self-employed and temporarily unemployed persons are eligible to receive this allowance. If work incapacity persists and prevents gainful employment, they are potentially eligible for a long-term Government-supported disability pension.

Statistical analysis

To identify associations between baseline parameters and loss of working hours, we included patients who were working at least one point in time over their follow up period and compared these to age-, gender- and time-matched Australian population data. To assess factors associated with permanent loss of employment, we included patients who were working at baseline. We utilised data for all those working until they ceased work (irrespective of age), if the reason for ceasing work was RA. Associations between disease and demographic factors and loss of working hours, were investigated using a random coefficients mixed model, with clustering over participant and time to account for correlated readings. Homoscedasticity and normality or residuals at each level were checked for model violation. For those who were working at baseline, time to permanent loss of employment was assessed using Cox proportional hazards, clustering over participant. The proportional hazards assumption was checked for model violation. Covariates in both models were age, symptom duration prior to diagnosis, gender, presence of rheumatoid factor (RF) or anti-cyclic citrullinated peptide (CCP), DAS28(ESR), modified HAQ, helplessness index, education status and baseline SvH score. A p-value <0.05 (two-tailed) was considered statistically significant and results are reported with 95% confidence intervals (CI) where appropriate. Differences between working and non-working population were calculated using Chi-squared, t-tests or Wilcoxon tests as appropriate. All analyses were conducted using STATA 13.1, StataCorp, Texas.

Data were available for 299 patients. Work data comprised 1562 observations. Follow-up was up to 14 years after commencing therapy, with a median of 3.7 years. Among 299 patients, 135 were working at baseline and 137 were working at any point. Of those working, 70% were female and 70% and 68% were seropositive for RF and anti-CCP respectively. Baseline DAS28 indicated moderate disease activity; the SvH score was >0 units in 60% and the baseline mHAQ was 0.65. At six months, 54% were EULAR good responders. The mean HWPW were 31.3 hours. Less than half had completed university or other tertiary education. Of those working, only 5 patients progressed to a biologic DMARD after a mean follow-up of 2.4 years.

Table 1. Baseline demographics[#] of those working.

	For association of loss of working hours (n=137)	For survival analysis (n=135)
Age, years, mean (SD)	45 (11)	46 (11)
Females (%)	96 (70)	92 (68)
RF positive (%)	96 (70)	94 (70)
Anti-CCP positive (%)	92 (68)	91 (68)
Symptoms prior to diagnosis, weeks, mean (SD)	21 (15)	20 (13)
DAS28, mean (SD)	5.07 (1.22)	5.04 (1.25)
mHAQ, mean (SD)	0.65 (0.51)	0.65 (0.52)
Total SvH x-ray score >0 (%)	62 (60)	60 (61)
Follow up duration, years, median (IQR)	3.7 (1.0-5.7)	3.5 (1.0-5.7)
Helplessness Index, mean (SD)	8.7 (4.7)	8.67 (4.82)
Education		
Primary/ secondary/ trade school (%)	71 (54)	71 (54)
University/ other tertiary (%)	60 (46)	60 (46)

[#] SD- standard deviation, RF- rheumatoid factor, CCP- cyclic citrullinated peptide, DAS28- disease activity score calculated using 28 joint count, mHAQ- modified health assessment questionnaire, SvH- van der Heijde modified Sharp score, IQR- inter-quartile range

Patients who were working at any point (n=137) were used to assess associations with loss of hours worked, while those who were working at baseline (n=135) were used in the survival analysis. Overall, 127 patients were common to both analysis sets. Demographic data are presented in **Table 1**.

Associations between loss of working hours and disease and demographic factors

Multivariable analysis (**Table 2**) revealed highly significant differences between working hours lost and baseline age with a loss of 0.41 hours (95% CI 0.14, 0.68) with each year of increasing age. Men worked 13 (95% CI 9.97, 19.99) hours per week more than women. Those with higher levels of education worked 5.84 (95% CI 1.18, 10.50) fewer hours but stayed in the work-force longer. There was no significant association between RF, anti-CCP, baseline DAS28, mHAQ, SvH score or helplessness index and loss of working hours.

Table 2. Associations of potential confounders[#] with loss of working hours.

	Univariable analysis (n=136)			Multivariable analysis (n=67)		
	β	(95% CI)	p value	β	(95% CI)	p value
Age (years)	-0.02	(-0.25, 0.22)	0.89	-0.41	(-0.68, -0.14)	**0.003**
Female gender	-12.20	(-17.49,-6.92)	**<0.001**	-12.98	(-19.99, -9.97)	**<0.001**
Education[†]	-4.85	(-9.16, -0.54)	**0.027**	-5.84	(-10.50, -1.18)	**0.014**
Time in study	-0.84	(-1.42, -0.27)	**0.004**	-0.63	(-1.22, -0.05)	**0.034**
Symptoms duration prior to diagnosis	-0.13	(-0.31, 0.04)	0.13	-0.15	(-0.33, 0.01)	0.073
RF positive	-2.32	(-7.95, 3.31)	0.42	1.65	(-4.95, 8.27)	0.49
Anti-CCP positive	-3.92	(-9.35, 1.50)	0.16	-5.24	(-11.67, 1.18)	0.11
DAS28, baseline	-1.85	(-3.94, 0.23)	0.08	-0.46	(-2.59, 1.66)	0.67
mHAQ, baseline	-4.46	(-9.43, 0.50)	0.08	-2.30	(-7.98, 3.37)	0.43
Baseline SvH score >0	-4.01	(-10.17, 2.14)	0.20	-0.39	(-6.04, 5.26)	0.89
Good EULAR responders[‡]	0.51	(-4.88, 5.90)	0.85	1.38	(-3.59, 6.35)	0.59
Helplessness index	-0.27	(-0.91, 0.36)	0.40	-0.47	(-1.10, 0.17)	0.15

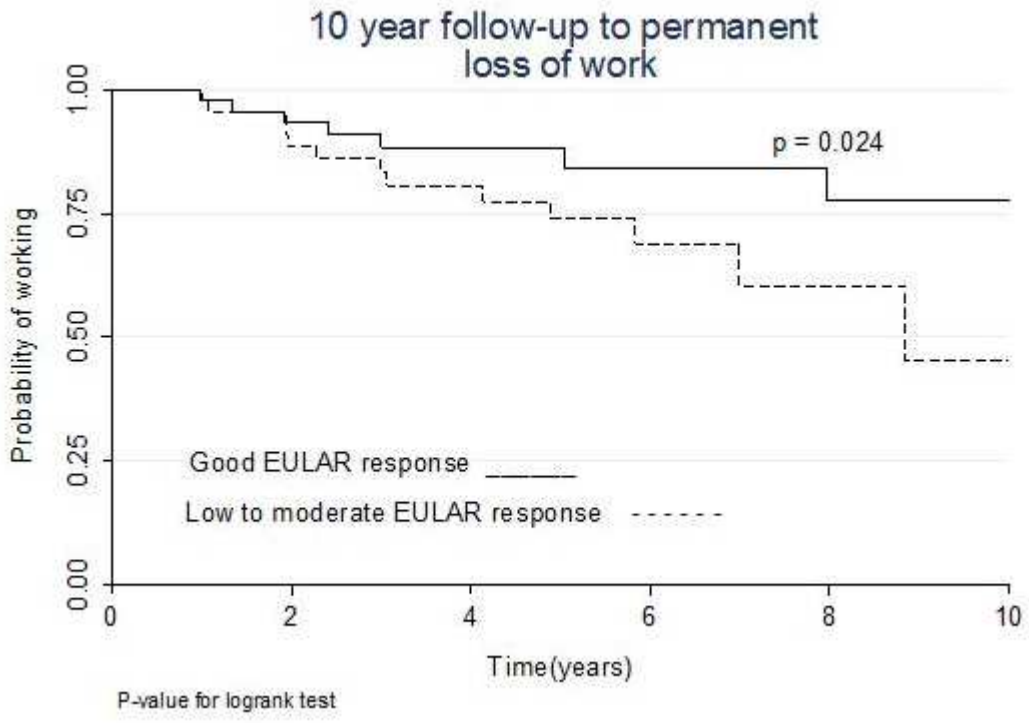
[#] RF- rheumatoid factor, CCP- cyclic citrullinated peptide, DAS28- disease activity score calculated using 28 joint count, mHAQ- modified health assessment questionnaire, SvH- van der Heijde modified Sharp score, IQR- inter-quartile range. † reference is primary/secondary/ TAFE vs University/ other. ‡ determined at 6 months

Neither baseline nor area-under-the curve for the first year for DAS28 (ESR) and mHAQ was associated with loss of working hours.

Relationship between treatment response, loss of employment and associated factors

The Kaplan-Meier survival estimate for loss of employment (**Figure 1**) revealed a significant difference between those who had a good EULAR response compared with those who did not. Among those who had a good response at 6 months, 77% were working at 10 years compared to 45% of moderate to low responders (p=0.024). The difference in the proportion still working between these two groups was apparent as early as two years after starting DMARD therapy and became more apparent over the next eight years. The 50% probability of being unable to work was reached at 9 years only in the moderate/ no EULAR responder group.

Figure 1. There was a significant difference between time to loss of employment in treatment responders and non-responders.



There was a trend towards an association between higher scores on the helplessness index and permanent loss of work (p=0.06). Multivariable Cox regression (**Table 3)** revealed an interaction between age and anti-CCP status. Increasing age was not associated with permanent loss of work for subjects who were anti-CCP negative (p=0.68). In contrast, permanent loss of employment and increasing baseline age were strongly associated for participants who were anti-CCP positive, with a hazard ratio (HR) of 1.24 (95% CI 1.07, 1.44). The association between permanent loss of employment and anti-CCP status varied with age at first presentation. For those who were over 54 years, the HR was 9.23 (1.04, 82.08) and increased with increasing baseline age. For those below 54 years, anti-CCP status was not associated with permanent loss of employment.

Work disability as compared to the general population

Over the study period, there was a loss of 0.63 (95% CI 0.05, 1.22) hours worked per year compared with 0.24 hours lost per year in an age, gender and time-matched general population during the same period.

The differences between those working and not working

A significant number of our patients were not working (n=70, 33%) despite being in the working age group. At baseline, those not working were older (52 *vs.* 45 years; p-value <0.001), had higher disease activity as measured by the DAS28 (5.58 *vs.* 5.07; p=0.006) andmHAQ scores (0.905 *vs.* 0.655, p=0.002) and had a higher helplessness index (16.16 *vs.* 13.70; p=0.004). Those not working also had lower levels of educational qualification achieved: only 18% had received university or other tertiary education as compared to 46% of those who were working (p<0.001). There was no significant difference between the two groups with respect to disease-related factors such as seropositivity for RF or anti-CCP, symptom duration prior to diagnosis, duration of follow up or the baseline SvH score.

Table 3. Cox regression analysis for associations of potential confounders‡ with loss of employment.

	Univariable analysis (n=133)			Multivariable analysis (n=72)		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Age						
Anti-CCP negative	0.98	(0.91, 1.06)	0.62	1.03	(0.87, 1.23)	0.68
Anti- CCP positive	1.09	(1.00, 1.21)	0.055	**1.24**	**(1.07, 1.44)**	**0.004**
Anti-CCP positive (at age = 54)#	**10.19**	**(1.69, 61.33)**	**0.011**	**9.23**	**(1.04, 82.08)**	**0.046**
University Education	1.92	(0.98, 8.33)	**0.054**	2.14	(0.50, 9.11)	0.30
Female gender	0.83	(0.33, 2.07)	0.69	1.55	(0.31, 7.84)	0.59
Symptoms duration prior to diagnosis	1.01	(0.99, 1.03)	0.28	1.02	(0.94, 1.11)	0.58
RF positive	1.71	(0.61, 4.74)	0.30	0.74	(0.19, 2.85)	0.66
DAS28, baseline	0.94	(0.65, 1.34)	0.73	0.83	(0.49, 1.40)	0.49
mHAQ, baseline	1.15	(0.62, 2.13)	0.64	1.08	(0.21, 5.49)	0.91
EULAR good responder	0.45	(0.18, 1.15)	0.09	0.22	(0.03, 1.91)	0.17
Baseline SvHS score >0	2.23	(0.75, 6.63)	0.15	1.55	(0.30, 7.84)	0.59
Helplessness index	1.06	(0.98, 1.15)	0.15	1.21	(0.98, 1.49)	0.06

‡ RF- rheumatoid factor, CCP- cyclic citrullinated peptide, DAS28- disease activity score calculated using 28 joint count, mHAQ- modified health assessment questionnaire, SvH- van der Heijde modified Sharp score. # At age >54 Anti-CCP is significantly associated with permanent loss of employment. At age <54 the association is non-significant.

DISCUSSION

Our study demonstrates that patients with early RA managed with a treat-to-target strategy maintain their work capacity, particularly if they are good EULAR responders. We also found a significant association between increasing age, female gender and lower levels of education and loss of working hours. Permanent loss of employment was significantly associated with anti-CCP seropositivity.

Among the studies that have compared RA-associated work disability with the general population,[410,415,417] very few studies utilised a treat-to-target treatment approach. Notable

examples include the FIN-RACo study that used combination (or single) conventional DMARD treatment with prednisolone (for 9 months or longer)[170] and the SWEFOT study that randomised methotrexate inadequate responders to infliximab or sulfasalazine plus hydroxychloroquine.[178] The FIN-RACo study found significantly lower work disability in those randomised to combination therapy vs. single drug therapy (32.2 days vs. 12.4 days per patient-observation year), as well as preservation of work capacity in those who were in ACR remission at 6 months [170,378]. This was particularly true for those receiving combination treat-to-target therapy and with good physician compliance with treat-to-target strategy.[418,419] In the SWEFOT study [178], mean work loss reduced from a median of 16 days per month to 4.9 days per month in the infliximab group and to 6.2 days per month in the combination DMARD group (a strategy similar to our initial therapy) over 21 months. Taken together, these results suggest that intensifying treatment improves work outcomes. However, the Fin-RACo study used long-term prednisolone. The high prevalence of comorbidities exacerbated by corticosteroid use means that it is preferable to avoid prednisolone in many individuals.

Our treatment strategy also entailed combination conventional DMARDs with a treat-to-target strategy but in contrast, oral corticosteroids were actively avoided. Despite this, the preservation of work ability at 5 years among good EULAR responders was similar if not slightly better (88% vs. 80%) to that achieved in the combination therapy arm of the FIN-RACo cohort and better than the single DMARD treatment arm (70%). The mean ages in the two studies were similar as was RF status.[170] Our patients had a shorter duration of symptoms prior to recruitment and better functional status at baseline which may portend a better prognosis. The SWEFOT study[178] also demonstrated the benefits of intensive therapy for work ability, although differing outcome measures and duration of follow-up make direct comparison with our cohort difficult. In addition, this population was slightly older, had more females and higher mHAQ scores at baseline.

The FIN-RACo study found work retention at 5 years was significantly better in the more intensively treated combination therapy group where permanent retirement from work was approximately 20%. This was similar to those who had a good EULAR response in our study (88%). The FIN-RACo study also identified significantly better long-term preservation of work ability among those who achieved remission or at least an ACR20 (American College of Rheumatology 20% response) response.[378] Our findings, with follow-up extending to 10

years are generally similar; good EULAR responders having 84% probability of preserved work capacity compared with 45% for moderate/ no EULAR responders.

Survival analyses from previous studies report that the 50% probability of being unable to work varies from 4.5 to 22 years, with a median of 13 years.[411] These older studies included cohorts who received conventional DMARDs, but did not employ a treat-to-target strategy. More recent data (from populations with early RA but not using a treat-to-target approach) show higher work survival rates,[171,172] with improvement in patients recruited in recent years.[171] Among our treat-to-target cohort, the 50% probability of being unable to work at 9 years was only reached in the moderate/ no EULAR responder group.

Neither the FIN-RACo nor the SWEFOT study specifically reported associations between disease or demographic parameters and work disability. This has been explored in other mainly conventional DMARD treated early RA cohorts[168,171,172,420,421] and systematic reviews.[167,411] Our findings are broadly consistent with these and confirmed the association of increasing age, longer duration of symptoms prior to diagnosis and lower education status with increased work disability. However, we did not find an association between mHAQ and work disability, nor did we observe the sharp initial fall in work-survival rates (in both EULAR good and moderate/no responders) seen in other studies. This may attest to the efficacy of combination treatment in the context of frequent (3-6 weekly) follow up visits and a treat-to-target approach.

Consistent with a previous cross-sectional study,[422] we identified a possible association between work disability and learned helplessness. Learned helplessness is perhaps more likely to occur among those slow to achieve good disease control and those less engaged in treatment and emphasizes the importance of achieving early treatment response and working in partnership with patients to achieve this.

There were similarities between those not working in our study and the unemployed (n=14) included in the FIN-RACo study; these individuals were less well educated and had previously had a more physically demanding job. The effects of seropositivity or radiographic damage were not reported.[170]

It is difficult to directly compare studies that assess work outcomes, not only due to the different treatment strategies applied and disease and work outcomes reported, but also

because of differences between labour markets and social security systems. [423] In general, the US and UK based studies have utilised self-reported disability, whereas the Northern European studies have used social security systems and public databases. The results of these studies have revealed consistent negative associations between work disability and physically demanding work type, higher HAQ scores, and personal factors.[172,411] Perhaps surprisingly, disease activity and radiographic correlations were inconsistently related.[167] This suggests that while EULAR and ACR responses may function as surrogate markers of work disability, the driving factors behind loss of work are not limited to disease activity alone.

Rates of work disability in RA vary across the world; even between developed countries, probably because of societal factors and differing social security systems. A study that included data from 32 countries confirmed the high rate of RA related work disability across nations, but strikingly, found that people in low-GDP countries continue to work at levels of clinical disease activity at which patients are work disabled in high-GDP countries, likely an influence of macro-economic, non-disease related factors. [424,425]

Australia has relatively strong disability supports so changes in work status among our cohort may be a more sensitive indicator of the challenges patients encounter. In 2008, a cross sectional study of Australian patients with established RA (median disease duration 10.5 years) found that 82% had ceased work because of their disease, with those in semi-skilled or unskilled jobs more likely to give up work. Younger patients, and those with less dependants, lower disease activity or HAQ scores were more likely to stay in the work force [415]. RA treatment has changed substantially over the last 15 years and the results from our study suggest that targeted treatment can improve work outcomes compared with historical cohorts. Our findings also suggest that a good EULAR response may be an appropriate treatment target to aid in improving work outcome.

This is the first Australian study, and one of only a few to analyse longitudinal work data from a closely followed treat-to-target inception cohort with age, gender and time-matched general population comparators. Furthermore, the quantification of work loss in terms of HWPW is potentially a more useful measure than days lost per year. Many patients reduce their working hours without reducing the total number of days worked and the average number of hours worked each day varies greatly, making this measure more sensitive to change.

The limitations of our study include a reliance on self-reported data (collected as a part of a wider study) and the lack of a control arm. Also, our study design limits results to associations rather than causative factors, and did not capture days of sickness due to RA. This was an open study design but the data were collected by a study metrologist and were not available to treating clinicians until after mid-2013. No treatment changes were made on the basis of work status.

In summary, our data suggest that intensive treatment is not only effective in achieving higher remission rates[379,398] but also translates into maintenance of work capacity over the longer term. This is particularly relevant for older patients or those who are anti-CCP positive. We found that good EULAR responders had significantly less long-term work disability than those with moderate or no EULAR response. Our findings suggest that increasing the proportion of those achieving a good EULAR response within 6 months of treatment is necessary if long-term work outcomes are to improve.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr Wechalekar had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Wechalekar, Proudman, EM Shanahan, Walker, Smith, Hill.

Acquisition of data. Wechalekar, Proudman, E Shanahan, Metcalf.

Analysis and interpretation of data. Wechalekar, Quinn, Lester, Proudman, Hill

CHAPTER 6. SYNOVIAL MEMBRANE RESPONSE TO COMBINATION CONVENTIONAL DMARD THERAPY IN EARLY RHEUMATOID ARTHRITIS

CHAPTER 7. THE ARBITRATE STUDY: A TRIAL ON **AR**THROSCOPIC SYNOVIAL **BI**OPSY DIRECTED **T**ARGETED BIOLOGIC THE**RA**PY VERSUS CONVENTIONAL **T**H**E**RAPY IN RHEUMATOID ARTHRITIS

CHAPTER 7. SUMMARY AND CONCLUSIONS

Rheumatoid arthritis (RA) is a relatively common autoimmune inflammatory disease affecting approximately 1-2% of the population. Its prevalence increases with age, approaching 5% in women above the age of 55 years, and with Australia's ageing population, it is likely that the prevalence will rise. The impact of RA on the affected individual and on society is profound. Although there have been substantial improvements in treatments over the last few decades sustained remission rates, particularly drug-free, even after use of combination disease-modifying anti-rheumatic drugs (DMARDs) adjusted according to disease activity or newer expensive biologic DMARDs, remain suboptimal.[454] The inability to reliably predict responses to a particular drug or drug combination perhaps explains the low rates of sustained remission despite a plethora of newer agents. It remains unexplained as to why some patients achieve remission whereas others continue to have active disease, despite current best-practice approaches. Furthermore, because treatments fail to achieve durable and sustained remission, disease-related morbidity remains substantial.

The aims of this thesis were to assess and develop measures to improve outcomes in early RA, a time when therapies are most likely to achieve sustained remission. We approached this question from several angles, beginning with an evaluation of the problems with the composite remission criteria currently in common use, that use abbreviated joint counts and thus omit the ankle and foot joints, common sites for initial joint involvement in RA. We explored whether ignoring ankle and foot disease in patients in apparent remission has an impact on long-term outcomes.

The second section of this thesis addresses whether current strategies used in treatment of early RA translate into personal and societal benefits in terms of preserved work outcomes. In this section, we examined the effects of a contemporary treat-to-target combination DMARD strategy on work disability, to tease out the relationship between response to treatment, and function. Because function has been robustly related to bone damage, in the latter half of this section, we explored whether the inclusion of bone biomarkers could explain radiographic progression despite apparent remission.

In the final section of this thesis, we explored novel ways of assessing and improving clinical response. In RA, the synovium probably best reflects the underlying cytokine and cellular mediators of inflammation and we were interested to explore the clinical applications of

arthroscopic synovial biopsy in early RA. We first undertook a pilot study and demonstrated that the synovium can be reliably sampled before and after treatment. This is followed by an ongoing study that involves rational drug selection based on the results of synovial biopsy in DMARD-naïve poor-prognosis patients with RA, a novel strategy that builds on current best-practice, with the aim of improving remission rates, achieving sustained remission and improving outcomes.

THE FOOT SYNOVITIS STUDIES

The goal of treatment in RA is to achieve remission. Ideally, this would imply a complete absence of disease activity: symptomatically, clinically, by imaging and by extension, lack of disease progression. Not only is complete remission hard to achieve, but it is also difficult to identify in routine clinical practice, due to the protean manifestations of the disease. Since no single measure can adequately capture RA disease activity (DA), various composite measures have been developed over the years; these measures assess a variable number of joints included in the DA score. Several of these, notably the DAS28,[66] SDAI and CDAI[68] utilise 28 joint counts. The newer Boolean-based 2011 ACR/EULAR criteria[69] also permit assessment of 28 joints, thus omitting joints of the feet. Foot synovitis is a component of active RA that is not necessarily measured well by other indices. This is particularly relevant in early RA, given that more than a third of patients have involvement of the foot joints prior to the hands, and ~90% of patients report painful ankles or feet at some point during the course of their disease.[391]

Our initial cross-sectional study on foot synovitis[82] assessed whether currently accepted criteria for disease activity and remission in RA (DAS28, SDAI, CDAI, ACR1987, ACR/EULAR 2011) that omit foot joint counts (or permit limited joint count assessment), underestimate DA by their inability to detect foot synovitis. We found that ongoing foot synovitis was present in >20% of patients meeting 28 joint count remission criteria and concluded that DA measures that omit foot joints do not provide an accurate reflection of an individual patient's disease activity. It follows that treatment decisions made solely on the basis of criteria that omit foot joint assessment may subject patients to ongoing joint damage (in contrast to the 1981 ACR or 2011 ACR/ EULAR remission criteria which use full joint counts).

The second foot synovitis study built upon our previous findings. We sought to confirm our findings longitudinally by analysing dynamic correlation between the ability of DA measures to 'capture' foot synovitis over 3 years. Our outcome measures included:

(i) Dynamic correlation between DA measures and foot synovitis

(ii) Radiographic progression, a robust longitudinal outcome measure,[392] which has been shown to occur more frequently at baseline and over time in comparison to hand joints in early RA,[393] and

(iii) The short-form SF-36 to capture the effects of foot synovitis on quality of life and activities of daily living such as climbing stairs, walking and recreational activity such as sports.

We found that all DA scores assessed (DAS28, SDAI and CDAI) showed a weak to moderate positive correlation with foot swollen joint counts (SJC) and tender joint counts (TJC) but despite the statistical significance of these correlations, the DA scores 'captured' less than 50% of the variation in foot SJC/TJC counts. This indicates that assessment of disease activity using these criteria is likely to be insufficient for detecting disease flares in the feet. Consistent with our previous cross-sectional findings, even the more stringent SDAI and CDAI remission criteria also failed to identify a significant proportion of patients (24% in SDAI and 25% in CDAI vs. 36% in DAS28 remission respectively) with ongoing foot synovitis. Not surprisingly, we found that remission in the presence of foot synovitis represented an unstable state, likely to relapse into active disease. We also found that being in sustained remission was strongly associated with the absence of radiographic progression. This, combined with the influence of foot synovitis on remission sustainability, further underscores the importance of assessing foot joints. The morbidity of foot synovitis was captured by SF-36 scores where we found foot synovitis to be independently associated with the physical functioning subscale of the SF-36, even after adjustment for disease activity. This second study confirmed and extended our previous findings concerning the underestimation of disease activity using DA measures that omit foot joints. When these criteria are used to define remission, a substantial proportion of patients have ongoing foot synovitis, which predicts relapse of standard DA measures, radiographic progression and worse functional outcomes over the longer-term.

The major limitation of these two studies was the absence of a specific measure of foot function (e.g. the Bristol Foot Score). Also, the number of patients in apparent remission with radiographic progression, was relatively small in this intensively treated cohort and this may have affected our results due to Type II error.

Nonetheless, our findings suggest that clinical trials should routinely use DA measures that include ankle/foot joints. From the perspective of the individual patient, it also remains important to adequately assess foot joints. Possible appropriate measures include the 1981 ACR criteria for remission or the 2011 ACR/EULAR criteria which utilise full joint counts. The original DAS also used full joint counts but was found to be too unwieldy in routine clinical practice. Abbreviated joint counts are undoubtedly more convenient in routine clinical practice and an alternative approach in the presence of time constraints may be to score ankle/foot joints as 'a block' with regards to presence or absence of synovitis. Detecting synovitis in the feet can be challenging and a further option would be to utilise a more sensitive measure such as ultrasound examination or MRI, to assess foot synovitis when clinically suspected. These alternative approaches could be the subject of a future prospective study.

ARTHRITIS AND WORK

There are limited longitudinal data, particularly in Australia[170,178] on the effects of a treat-to-target strategy using combination conventional DMARDs, without the use of corticosteroids and biologic DMARDs. [415] In this study, we investigated work disability in patients with early RA treated with combination conventional DMARD therapy, using a treat-to-target strategy without initial oral corticosteroids or biologic DMARD. Our findings revealed that good EULAR responders (as measured at 6 months) were more likely to be working at 10 years as compared to those with moderate/no EULAR response. This difference became apparent as early as 2 years and more pronounced over the next 8 years after commencing DMARD therapy. We also found hours-worked-per-week in our cohort to be generally preserved as compared to an age, gender and time-matched general population. We did not find a significant association between mHAQ and work disability, nor did we observe the sharp initial fall in work-survival rates (in both EULAR good and moderate/no responders) seen in other studies. This may attest to the efficacy of combination treatment in the context of frequent (3-6 weekly) follow up visits and a treat-to-target approach. Our study has

important economic and therapeutic considerations, given the relatively low cost of DMARD therapy as compared to biologic DMARD therapy. Many patients have comorbidities where the use of corticosteroids is relatively contraindicated and it is reassuring that the low levels of corticosteroid use in our cohort did not translate to worse work outcomes.

Our study had significant limitations, in particular a reliance on self-reported data and we did not capture days of sickness due to RA. Another limitation was the lack of a control arm. Although we incorporated the helplessness scale, we did not have a formal measure of the psychological impact of disease, known to have a significant impact on ability to work in RA. In addition, the numbers of patients still in the study at 10 years was relatively small, and this may have skewed the results.

Our study underscores the importance of early diagnosis of RA, and early intensive therapy, both of which have been shown to improve disease related outcomes, and should potentially, translate into better work outcomes. In particular, it emphasises that those who do not have a good EULAR response early in the course of treatment are likely to have worse work-outcomes. It is in this population that alternate treatment strategies should probably be pursued to achieve remission. We postulate that including even more stringent measures to capture ongoing foot synovitis may also further improve work outcomes.

MARKERS OF BONE DAMAGE

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THE ARBITRATE STUDY

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This thesis has explored ways to improve outcomes in early RA. We assessed the limitations of current DA measures and found that many of the commonly used scores fail to adequately capture ongoing disease activity in patients with foot synovitis, a common cause of morbidity in RA. We postulate that routine inclusion of DA measures that include foot joints may improve assessment of DA and allow escalation of therapy if indicated, in order to improve disease outcomes. We also identified that work outcomes are better in those achieving a good EULAR response providing further evidence of the benefits of achieving remission, particularly early in the disease. We explored bone biomarkers to capture the uncoupling that is recognised to occur between disease activity and bone damage. Our findings are complex and highlight the fact that these biomarkers must be considered in conjunction with disease activity and time course of the disease to allow meaningful interpretation. Finally we demonstrated in a pilot study that serial synovial arthroscopic biopsy can be successfully employed in patients with early arthritis and we discuss the ongoing ARBITRATE study, initiated to address whether synovial biopsy and targeted therapy may improve outcomes in early disease.

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