THE EPIDEMIOLOGY OF MUSCULOSKELETAL INFLAMMATION

by

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For my children, Helena Luisa and Paola

For my parents
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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by progressive joint destruction often resulting in functional disability and increased mortality. With appropriate therapies, it is possible to delay or even prevent evolution of patients into RA and/or induce remission. Thus the identification of individuals not only early in the disease course but at risk of developing RA is important. Following on this concept, we investigated the presence of systemic autoimmunity among individuals at risk of RA based on environmental exposures, and conducted a meta-analyses showing an early therapeutic window of opportunity associated with sustained benefit on disease progression and structural damage. Since time matters, we examined both the recent criteria performance and the role of musculoskeletal ultrasound as tools to identify RA early in the disease course.

One of the therapeutic goals in RA is the prevention of radiologically evident joint destruction, thus we evaluated a novel scoring method to assess radiological disease progression. We also examined the impact of inflammation on RA-associated collateral damage, including cardiovascular disease and bone loss. We observed within the epidemiology of musculoskeletal involvement that chronic inflammation in any one tissue clearly impacts the overall health status and disease susceptibility of the whole body.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AATD</td>
<td>α1-antitrypsin deficiency</td>
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<td>ACPA</td>
<td>anti-citrullinated peptide antibodies</td>
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<td>ACR</td>
<td>American College of Rheumatology</td>
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<td>AIMS</td>
<td>Arthritis Impact Measurement Scale Health Status Questionnaire</td>
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<td>APRIL</td>
<td>a proliferation-inducing ligand</td>
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<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BlyS</td>
<td>B-lymphocyte stimulator</td>
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<td>BMD</td>
<td>bone mineral density</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CCP</td>
<td>cyclic citrullinated peptide</td>
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<td>CD</td>
<td>cluster of differentiation</td>
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<td>CDAI</td>
<td>clinical disease activity index</td>
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<tr>
<td>CEP-1</td>
<td>citrullinated enolase peptide-1</td>
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<tr>
<td>CHF</td>
<td>chronic heart failure</td>
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<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>cit-fib</td>
<td>citrullinated fibrinogen</td>
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<tr>
<td>cit-vim</td>
<td>citrullinated vimentin</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<tr>
<td>COX-2</td>
<td>cyclooxygenase-2</td>
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<td>CParg</td>
<td>negative control peptide for CCP</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CTLA4</td>
<td>cytotoxic T-lymphocyte antigen 4 gene</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DAS28</td>
<td>disease activity scores of 28 joints</td>
</tr>
<tr>
<td>DMARDs</td>
<td>disease-modifying antirheumatic drugs</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>EBV</td>
<td>Epstein Barr virus</td>
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<td>EGA</td>
<td>evaluator global health assessment</td>
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<tr>
<td>ESR</td>
<td>erythrocyte sedimentation rate</td>
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<tr>
<td>EULAR</td>
<td>European League against Rheumatism</td>
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<tr>
<td>Fcγ receptors</td>
<td>Fc receptors for IgG</td>
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<td>FDA</td>
<td>Food &amp; Drug Administration</td>
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<tr>
<td>fib</td>
<td>fibrinogen</td>
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<td>FLS</td>
<td>fibroblast-like synoviocytes</td>
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<td>GFR</td>
<td>glomerular filtration rate</td>
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<td>GM-CSF</td>
<td>granulocyte-macrophage colony-stimulating factor</td>
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<td>HAQ</td>
<td>Stanford Health Assessment Questionnaire</td>
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<td>HDL</td>
<td>high-density lipoprotein</td>
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<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>HSP</td>
<td>heat-shock proteins</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<td>IL</td>
<td>interleukin</td>
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<td>JAK</td>
<td>Janus kinase</td>
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<td>JSN</td>
<td>joint space narrowing</td>
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<td>JSW</td>
<td>radiographic joint space width</td>
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<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinases</td>
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<td>MCP-1</td>
<td>monocyte chemoattractant protein-1</td>
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<td>M-CSF</td>
<td>macrophage colony-stimulating factor</td>
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<td>MCV</td>
<td>mutated citrullinated vimentin</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>miRNAs</td>
<td>micro ribonucleic acid</td>
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<td>MMPs</td>
<td>matrix metalloproteinases</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MTP</td>
<td>metatarsophalangeal joints</td>
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<td>NF-kB</td>
<td>nuclear factor-kappa B and activator protein-1</td>
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<td>NF-kB</td>
<td>nuclear factor</td>
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<tr>
<td>NLRs</td>
<td>nucleotide-binding oligomerization domain (NOD)-like receptors</td>
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<tr>
<td>NOD</td>
<td>nucleotide-binding oligomerization domain</td>
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<td>NSAIDS</td>
<td>non-steroidal anti-inflammatory drugs</td>
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<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PADI</td>
<td>peptidyl arginine deiminase</td>
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<td>PADI-4</td>
<td>peptidyl arginine deiminase type IV</td>
</tr>
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<td>PD</td>
<td>periodontal disease</td>
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<td>PDGF</td>
<td>platelet-derived growth factor</td>
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<td>PGA</td>
<td>patient global health assessment</td>
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<td>PPAD</td>
<td>microbial peptidyl arginine deiminase</td>
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<tr>
<td>PTPN22</td>
<td>protein tyrosine phosphatase N22</td>
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<tr>
<td>RA</td>
<td>rheumatoid arthritis</td>
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<tr>
<td>RADAI</td>
<td>rheumatoid arthritis disease activity index</td>
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<td>RADAR</td>
<td>rapid assessment of disease activity in rheumatology</td>
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<tr>
<td>RANKL</td>
<td>receptor activator of NF-kB ligand</td>
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<tr>
<td>RAPID3</td>
<td>modified routine assessment of patient index data</td>
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<tr>
<td>REP-1</td>
<td>negative control peptide for CEP-1</td>
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<tr>
<td>RF</td>
<td>rheumatoid factor</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDAI</td>
<td>simplified disease activity index</td>
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<td>SE</td>
<td>shared epitope</td>
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<td>SJC28</td>
<td>swollen joint counts of 28 joints</td>
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<tr>
<td>SNPs</td>
<td>single-nucleotide polymorphisms</td>
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<td>STAT4</td>
<td>signal transducers and activators of transcription 4</td>
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<tr>
<td>SUA</td>
<td>serum uric acid</td>
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<tr>
<td>Th17</td>
<td>17 helper T cells</td>
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<tr>
<td>TIMPs</td>
<td>tissue inhibitors of metalloproteinases</td>
</tr>
<tr>
<td>TJC28</td>
<td>tender joint counts of 28 joints</td>
</tr>
<tr>
<td>TLRs</td>
<td>toll-like receptors</td>
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<tr>
<td>TNF-α</td>
<td>tumour necrosis factor-α</td>
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<tr>
<td>TRAF1-C5</td>
<td>TNF receptor-associated factor 1 and complement component 5</td>
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<tr>
<td>VAS</td>
<td>visual analogue scale</td>
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<tr>
<td>vim</td>
<td>vimentin</td>
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<tr>
<td>VSMC</td>
<td>vascular smooth muscle cell</td>
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A. Purpose and outline

Inflammation is the main pathologic process involved in many chronic disorders that affect several organs and systems, such as the autoimmune inflammatory rheumatic diseases, in particular rheumatoid arthritis (RA), which often results in functional disability and impaired quality of life. RA is characterized by increased mortality and significant morbidity including several coexisting conditions such as periodontal disease, accelerated atherosclerosis and bone loss. Substantial data demonstrates that essentially all chronic diseases are related through common inflammatory mechanisms and processes and that chronic inflammation in any one tissue impacts the overall health status and disease susceptibility of the whole body. However, when and where RA starts remains unknown.

The purpose of this thesis is to provide an overview of RA within the epidemiology of musculoskeletal involvement, with a focus on the phases of RA and the relevance of time in the management of RA, the associations and potential risk factors for RA, the tools used to assess disease prediction and progression, and the role of chronic inflammation as the common theme linking the spectrum of RA-coexisting conditions.

The relevance of time for the burden of RA is reviewed in paper 1\(^1\). Within the pathogenesis of RA, we investigated associations and potential risk factors for RA (papers 2-5). We first present a systematic review on the association between the rheumatic diseases and periodontal disease (paper 2)\(^2\), and we examined the link between rheumatoid arthritis in particular, and periodontal disease in a population-based study (paper 3)\(^3\). We then investigated the presence of systemic autoimmunity among individuals at risk of RA, based on their environmental exposures, such as periodontal disease (paper 4)\(^4\), and established lung disease (paper 5)\(^5\), either due to a genetic deficiency (i.e. α-1 antitrypsin deficiency) or to smoking, which is an established risk factor for RA.
Early treatment improves outcomes. We show in a meta-analysis of observational studies and randomized controlled trials that there is a critical period during which antirheumatic therapy should be initiated, a therapeutic window of opportunity early in the course of RA, associated with sustained benefit on disease progression and structural damage (paper 6)\(^6\). However, the timely initiation of anti-rheumatic therapy demands an early classification or diagnosis of RA, or of early arthritis at risk of developing into persistent and erosive disease. To help identify individuals early in the course of RA, we investigated the role of musculoskeletal ultrasound as a predictor of outcome in people with arthritis at the very early stages of the disease course (paper 7)\(^7\), and examined the performance of the recent 2010 ACR/EULAR criteria for classification of RA in a cohort of patients with very early synovitis (paper 8)\(^8\).

The goal of therapy is to improve the symptoms and signs of RA and prevent radiologically evident joint destruction or functional loss. In the next few papers, we examined the impact of inflammation at the level of the joint and investigated the performance and both, patient repositioning and examiner reproducibility, of an automated computer-based scoring method to assess radiological structural damage and disease progression in RA (papers 9-11)\(^9\)\(^-\)\(^11\).

The following papers addressed some of the systemic consequences of chronic inflammation. We first focused on the role on inflammation in the established increased risk of cardiovascular disease in RA (papers 12-14)\(^12\)\(^-\)\(^15\). We investigated antioxidants and other novel and traditional risk factors for cardiovascular disease in RA, and assessed the relationship between serum uric acid, long implicated in cardiovascular morbidity, and inflammation in people with and without RA. Finally, we examined whether systemic inflammation has an impact on bone in a large epidemiologic study (paper 15)\(^15\).
B. Summary sheet

C. Introduction

Rheumatoid arthritis

Rheumatoid arthritis (RA) is the most common immune mediated rheumatic chronic inflammatory disease affecting about 1% of the population\(^\text{16}\). Women are affected two to three times more often than men. RA can occur at any age. The peak onset is between 50 and 75 years of age, and the prevalence is 5% among women over age 65. The lifetime risk of RA in adults is about 4% in women and 2% in men\(^\text{17}\). RA is characterized by synovial inflammation and progressive cartilage and bone destruction that often results in substantial pain, disability, loss of function and other comorbidities. RA clinical features include pain, swelling, stiffness and a limited range of motion in the joints involved. Despite recent advances in treatment modalities used to modify the course of the disease, RA is associated with long-term comorbidity\(^\text{18-20}\) and accelerated mortality. Uncontrolled disease may confer a large socioeconomic and psychosocial burden as a consequence of joint destruction and deformity at the individual level, with loss of work productivity\(^\text{21,22}\). Given the systemic nature of the disease, RA clinical manifestations are not limited to the joints and range from constitutional symptoms to organ involvement including a wide variety of extra-articular manifestations such as subcutaneous nodules, pulmonary nodules, interstitial-lung disease, vasculitis, intestinal fibrosis, lung involvement, pericarditis, and eye involvement (e.g. episcleritis, uveitis). Furthermore, chronic inflammation has detrimental effects not only as a result of direct damage but also due to collateral damage in other systems and tissues resulting in comorbid diseases\(^\text{18-20}\). RA is associated with significant comorbidity including systemic features with accelerated atherosclerosis, pulmonary, skeletal, and psychological involvement, and other diseases such as periodontitis. Structural damage occurs very early in the course of the disease and is closely related to unsuppressed disease activity.
The clinical presentation of RA is very heterogeneous. Among patients presenting with symptoms of early inflammatory arthritis, 60% have a self-limiting disease while 40% develop a chronic persistent arthritis. The diagnosis/classification of RA relies upon the use of clinical criteria\textsuperscript{23,24}, as no diagnostic serological test is available. RA features include autoantibody production such as rheumatoid factor (RF) and the highly RA-specific anticyclic citrullinated antibodies (ACPA), which define disease subgroups with distinct prognosis\textsuperscript{25}. Ultrasound or magnetic resonance imaging may detect joint inflammation and structural changes early in the disease course, while radiographs of the hands and feet may be normal in up to 80% of patients with early RA. The last two decades have seen important advances in understanding the incidence, treatment and outcome of patients with early rheumatoid arthritis. With appropriate therapies, it is possible to delay or possibly even prevent evolution of patients with undifferentiated arthritis into rheumatoid arthritis (RA) and/or induce remission in a substantial proportion of patients with RA. Furthermore, preventing the burden of RA may be best managed by identifying patients at risk of developing RA and intervening very early\textsuperscript{26,27}. However, when and where RA starts remains unknown, and as clinicians, we are still far from being able to produce an individualised treatment plan for each patient, partly because current prediction tools for RA are not perfect.

The purpose of this thesis is to provide an overview of RA within the epidemiology of musculoskeletal involvement, with a focus on the phases of RA and the relevance of time in the management of RA, the associations and potential risk factors for RA, the tools used to assess disease prediction and progression, and the role of chronic inflammation as the common theme linking the spectrum of RA-coexisting conditions.
D. Pathogenesis of rheumatoid arthritis

Although widely recognized as an autoimmune disease, the cause of RA remains unknown. A range of stimuli have been implicated as possible primary causes that activate the immune response. These include environmental factors, hormonal factors, connective tissue proteins, immunoglobulins and infectious agents, such as bacteria or viruses, which may trigger RA in those with a genetic susceptibility. The clinical syndrome of polyarticular synovitis is initiated by multiple factors or a combination thereof in genetically susceptible hosts, and once started, the process becomes self-perpetuating and expands to the whole body (Figure 1).

Figure 1. Genes and environment in the development of rheumatoid arthritis
1. **Genetic and environmental factors**

1.1. **Genetics**

Several genetic loci have been linked with RA susceptibility and severity. Twin studies, with concordance rates of 15-30% for monozygotic and 5% for dizygotic twins imply a genetic susceptibility\(^2^8\). Genetic factors contribute between 53-65% of the risk of RA\(^2^8\). The strongest link between a genetic factor and RA is the association with an epitope in the third hypervariable region of the human leukocyte antigen (HLA)-DRB1 molecule (amino acids 67-74) known as the shared epitope (SE)\(^2^9\), particularly in seropositive or ACPA positive RA. The SE also confers disease severity as SE carriage (1 or 2 alleles) is associated with erosions\(^3^0\). Genes outside of the major histocompatibility complex (MHC) region are associated with RA. Several genes with single-nucleotide polymorphisms (SNPs) have been linked to ACPA positive RA, mediated by T-cell pathways including stimulation, activation, and functional differentiation (e.g. protein tyrosine phosphatase N22 (PTPN22) and cytotoxic T-lymphocyte antigen 4 gene (CTLA4)), the nuclear factor kB (NF-kB) dependent signalling pathway (e.g. TRAF1-C5 and c-REL), cytokine induced signals (STAT4), or other pathways\(^3^1\), including PADI-4 gene (peptidyl arginine deiminase type IV), Fc\(\gamma\) receptors (Fc receptors for IgG), CD40 and several cytokines, chemokines and cytokine-receptor loci.

Disease risk may be increased by gene-gene interactions compared to the risk conferred by any given gene\(^3^2\). The majority of genetic variants confer risk to ACPA-positive RA while other variants may be restricted to ACPA-negative RA, allowing a subclassification of RA\(^3^3\). A gene-environment interaction between smoking and HLA-DRB1 (SE) alleles has been identified in ACPA-positive RA. The genetic factors involved in ACPA negative RA are less well known and involve different pathways, such as interferon regulatory factors, lectin-binding proteins and different HLA alleles (e.g. HLA-DRB1*03)\(^2^5\).
1.2. Epigenetics

Epigenetics, defined as inherited changes in gene expression that are not encoded in the DNA sequence itself, have been recognized as important factors in controlling the expressed genome via gene transcription. Epigenetic modifications can influence disease susceptibility and severity in RA\textsuperscript{34-38}. Major epigenetics modifications include DNA methylation, histone modification, microRNA activity (miRNAs) and other post-translational processes, that directly influence genes involved in inflammation and tissue destruction. Importantly, the epigenome is influenced by environmental factors through life\textsuperscript{38}.

1.3. Smoking

Smoking is a strong environmental risk factor for the development of seropositive and ACPA positive RA, with a clear dose-response relationship. RA risk increases after 10 pack-years of smoking and remains elevated up to 20 years after cessation\textsuperscript{39}. Smoking not only boosts the risk of developing RA among susceptible individuals, it can also increase disease severity and reduce the clinical effectiveness of medications used to treat RA. Strong combined gene-environment effects have been observed, with markedly increased risks of ACPA-positive RA among SE homozygotes who were heavy smokers (OR 53), heavy coffee drinkers (OR 53), or oral contraceptives users (OR 45)\textsuperscript{40}. Smoking in and of itself is of interest as a risk factor for autoimmunity\textsuperscript{41}. Citrullination is a post-translational modification of proteins within the context of inflammation\textsuperscript{42}, recognized to occur in the lung of smokers, and thought to influence occurrence of antibodies directed against citrullinated proteins\textsuperscript{43}. Smoking has been shown to contribute to citrullination of self-peptides and anti-citrulline autoimmunity\textsuperscript{44}. Genetic susceptibility factors for RA and smoking are associated mainly with development of ACPA positive RA and linked to the SE, as well as smoking, PTPN22 and the gene BRD2, with an antibody response to the citrullinated α-enolase peptide-1 (CEP-1)\textsuperscript{45}. 
1.4. Infectious agents

Infectious agents have been implicated with RA, including virus (Epstein Barr virus (EBV), cytomegalovirus), retrovirus, *Escherichia coli*, proteus species, and their products such as superantigens and heat-shock proteins (HSP), which share antigenic determinants with host proteins, resulting in the development of cross-reactive antibodies that may induce an autoimmune molecular mimicry response, e.g. two proteins that possess the shared epitope are EBV gp110 and *Escherichia coli* dnaJ, which is an antigenic heat-shock protein\(^46\). Infectious agents of the oral cavity have been suggested to contribute to RA aetiology, including periodontal bacteria\(^47\)\(^-\)\(^49\). Studies have demonstrated an antibody response against oral anaerobic bacteria in serum\(^49\)\(^50\) and synovial tissue\(^47\), as well as presence of oral bacterial DNA in the serum and synovial fluid of patients with RA\(^48\). *Porphyromonas gingivalis* is a common pathogen in periodontal disease that has a microbial peptidyl arginine deiminase (PPAD) enzyme\(^51\)\(^52\), which has been shown to citrullinate proteins\(^53\). Thus, it is conceivable that periodontal disease is associated with RA and may have a causal role in the etiology of RA.

1.5. Periodontal disease

Periodontal disease (PD: periodontitis) is a chronic inflammatory disease characterized by loss of the periodontal ligament and alveolar bone, and is a major cause of tooth loss. Chronic periodontitis is arguably the most prevalent chronic inflammatory disease of humans. A growing number of clinical studies have suggested associations between chronic periodontitis and various rheumatic diseases, in particular RA. Such an association may be very important from a clinical and public health perspective for several reasons. Firstly, there are several processes through which chronic periodontitis may be part of a causal pathway in the pathogenesis and/or activity status of rheumatoid arthritis. If proven, given the high prevalence of chronic periodontitis, a large proportion of RA incidence and/or morbidity
could be attributable to chronic periodontitis. Importantly, chronic periodontitis would represent a modifiable risk factor as very effective treatments for chronic periodontitis are available. Secondly, chronic periodontitis would contribute to morbidity in patients with RA. Periodontitis is a leading cause of tooth loss in adults, which has important clinical consequences, including nutritional status and quality of life. Furthermore, chronic periodontitis is associated with an increased incidence of coronary heart disease and stroke, and this association may at least in part be causal.

1.5.1. **Association between periodontitis and the rheumatic diseases**

We conducted a systematic review of the literature on the association between rheumatic diseases and chronic periodontitis (paper 2). Studies identified by searching PubMed database and published until November 2008 were considered. We also performed a hand search on reference lists of original articles as well as conference proceedings. We identified that published studies vary widely with respect to design, setting, and methods used to ascertain associations between rheumatic diseases and periodontitis. The majority of studies are relatively small prevalence case-control studies. Frequently, control subjects were volunteers recruited from the staff working at the study centres (e.g. university/hospital staff) or patients attending dental clinics raising concerns regarding study validity. The majority of oral care is administered in the primary care sector, and patients attending dental clinics or hospitals are unlikely to be representative of the general population. This may have resulted in over- or underestimation of the association between rheumatic disease and periodontitis/tooth loss. Another difficulty when evaluating the evidence for an association between rheumatic disease and periodontitis relates to lack of consistent criteria used to define a “case” of periodontitis. Periodontitis can be assessed clinically by measuring periodontal probing depth and attachment levels at various sites of the dentition or by measuring alveolar bone loss from
radiographs. Definitions of periodontitis in clinical research are based on either measure or on combinations thereof and vary widely between studies. Attempts to define case definitions of periodontitis for clinical studies (similar to the ACR criteria for RA) have only recently been made and have not yet been universally adopted. In addition, some studies have looked at serological markers of periodontitis (e.g. antibodies to periodontal pathogens), which may not correlate well with the clinical phenotype. Furthermore, some studies have used self-reported markers of periodontitis, which frequently lack validity. The majority of studies employed ACR criteria for defining RA; however, some studies used self-reported RA, which is notoriously inaccurate (specificity of 6%).

1.5.2. Link between rheumatoid arthritis and periodontal disease

We conducted a population based study based on the US National Health and Nutrition Examination Survey (NHANES III)\(^3\). RA was based on the ACR1987 criteria. Periodontitis was defined based on a standardized clinical examination by calibrated dental examiners. Participants with 4 out of 6 ACR criteria evaluated had a 4-fold increase in the odds of periodontitis (OR 4.1; 95% CI 1.3-13.1), after adjusting for age, gender, race/ethnicity, and smoking. RA was also associated with a 3-fold increase in the odds of edentulism (complete tooth loss) controlling for age, gender, race/ethnicity, and smoking. This association was particularly strong for those with seropositive RA (OR 4.5, 95% CI 1.2, 17), and was largely preserved with further adjustment for a number of potential confounders including education, income, diabetes, bone mineral density, BMI, and physical activity.

Several biologically plausible causal and non-causal mechanisms may account for this association (reviewed in paper 2)\(^2\) as illustrated in Figure 2. Recent evidence suggests that periodontitis may indeed be a causal factor in the initiation and maintenance of the autoimmune inflammatory response in RA. If so, chronic periodontitis may represent an
important modifiable risk factor for RA. In addition, patients with RA may be at increased risk for periodontitis and tooth loss through various mechanisms. Additional non-causal pathways include genetic, environmental and behavioural exposures common to both conditions. Susceptibility to chronic inflammatory diseases, including periodontitis and RA, is determined by the interplay between genetic factors and environmental exposures, which could represent common risk factors for both disease, and thus confound the association between RA and periodontitis (non-causal pathways). Periodontitis could be a causal factor in the pathogenesis of RA through various pathways, including periodontal pathogens and Porphyromonas gingivalis in particular. Conversely, PD may predispose to RA (Figure 2).

**Figure 2. Possible pathways in the association between RA and periodontal disease**
A common periodontal pathogen, *Porphyromonas gingivalis*, expresses a peptidyl arginine deiminase (PAD) enzyme\(^{51,52}\) that citrullinates mammalian proteins\(^ {53}\). Thus, individuals with periodontitis are exposed to citrullinated antigens that may become systemic immunogens \(^{54}\). Subjects with periodontitis may have positive ACPA in the absence of rheumatoid arthritis\(^ {55}\). Antibody titres to *P. gingivalis* have been correlated with anti-citrullinated protein antibodies (ACPA) in subjects with RA\(^ {50}\). An immunodominant epitope in citrullinated α-enolase (antibodies to citrullinated α-enolase are specific for RA) has been identified. The sequence similarity and cross-reactivity with bacterial enolase from *P. gingivalis* may indicate a role for this periodontal pathogen in priming inflammation in RA\(^ {56}\).

### 1.6. Autoantibodies

Rheumatoid factor (RF), an auto-antibody reacting with the Fc portion of immunoglobulin G (IgG), is present among 70-80% of patients with RA and about 10-20% of the general population. RF is associated with disease severity and extra-articular manifestations, but it is not specific to RA as it can be found in other chronic inflammatory conditions. More specific tests for RA have been developed over recent years, encompassing a range of antibodies against cyclic citrullinated peptides and proteins (CCP) under the umbrella term ACPA. ACPA may have different reactivities, including antibodies against CCP, mutated citrullinated vimentin (MCV), citrullinated α-enolase peptide-1 (CEP-1), citrullinated fibrinogen, citrullinated fibronectin and citrullinated collagen II. The presence of both RF and ACPA antibodies has a high specificity for the development of RA and actually precede the appearance of clinically identifiable arthritis by several years\(^ {57-59}\). This suggests that the initial immune dysregulation in RA occurs years prior to symptoms onset, although how this develops is yet unknown.
Citrullinated proteins have been identified in different types of inflammatory arthritis, as well as at other sites of inflammation. However, the development of ACPA appears to be specific to RA and is predictive of RA disease severity and clinical outcome\textsuperscript{60}. Since ACPA appear very early in the course of the disease, their detection is important to identify the most destructive form of RA, allowing early combination therapy to prevent irreversible structural damage\textsuperscript{60}. ACPA positive forms of RA may represent a clinically and pathologically distinct subpopulation. Two main ACPAs are used in clinical practice, anti-cyclic citrullinated peptide (anti-CCP) and anti-mutated citrullinated vimentin (anti-MCV) antibodies. Both are markers of RA disease severity\textsuperscript{61,62} and extra-articular disease\textsuperscript{63}, particularly lung disease\textsuperscript{64}. The lung has been suggested as a site of initial RA-related immune dysregulation based on the occurrence of lung involvement in early RA, as well as the association with smoking, and occupational exposures such as silica dust, mineral oil, and air pollution\textsuperscript{65-86}, which suggests that respiratory exposures activating the immune system may lead to the development of RA. Smoking has been shown to contribute to citrullination of self-peptides and ACPA autoimmunity\textsuperscript{43,44}. The combined effect of shared epitope, PTPN22 and smoking showed a strong association with the anti-CEP-1 positive subset compared with the anti-CEP-1–negative / ACPA positive subset, suggesting that CEP-1 is a specific citrullinated autoantigen that links smoking to genetic risk factors in the development of RA\textsuperscript{45}. The stress of mucosal barriers may trigger post-translational modifications mediated by peptidyl arginine deiminase enzymes such as PAD4, that result in citrullination of proteins or peptides present in mucosal tissues. These modified proteins or peptides may result in ACPA response and reactivity triggering autoimmunity. To investigate this, we conducted two prospective studies in samples of non-RA patients potentially at risk of developing RA, based on their exposures and/or diagnoses. Based on recent EULAR recommendations on the specific preclinical
phases up to the development of RA\textsuperscript{87}, we included individuals classified as (b) (i.e. with environmental risk factors for RA), and tested whether these individuals had progressed from (b) to (c) (i.e. whether these individuals at risk for RA had developed systemic autoimmunity associated with RA).

1.6.1. Role of periodontitis in the induction of RA-related autoimmunity

This study tested the autoantibody repertoire in periodontal disease (paper 4)\textsuperscript{4}, following on our previous observations based on our population-based study, in which we observed a significant four-fold increase in the odds of periodontitis in people with RA (paper 3)\textsuperscript{3} and our systematic review of the literature suggesting an association between periodontitis and RA (paper 2)\textsuperscript{2}. Further, \textit{P gingivalis} is a common periodontal pathogen with a PPAD enzyme, which is unrelated to PAD enzymes in vertebrates\textsuperscript{89}. Nonetheless, microbial PAD activity deaminates arginine in fibrin found in periodontal tissue\textsuperscript{51} and citrullinates mammalian proteins\textsuperscript{53}, thus it is conceivable that PD may have a causal role in the etiology of RA. We hypothesized that RA-specific autoimmunity may be generated in the gums and that periodontal citrullination might be a relevant process generating autoantibodies in PD. The purpose of this study was to determine the immune reactivity to ACPA and their uncitrullinated control peptides in patients with periodontitis diagnosed by periodontal and/or radiological examination. Serum samples were tested for the following ACPA by ELISA: anti-cyclic citrullinated peptide (anti-CCP), anti-mutated citrullinated vimentin (anti-MCV), anti-citrullinated \(\alpha\)-enolase peptide-1 (CEP-1), vimentin (cit-vim), fibrinogen (cit-fib) and their uncitrullinated forms CParg (negative control peptide for CCP) REP-1, vim and fib). Multiple regression models were used to evaluate differences in ACPA response between individuals with and without periodontitis, adjusting for potential confounders. We analysed 96 patients with and 98 without periodontitis, none of whom had RA at inclusion. The
prevalence of anti-CCP and anti-MCV was around 1% in both PD and non-PD subjects. Periodontitis, compared with non-PD, was associated with a higher frequency of positive antibodies to CEP-1 (12% and 3%, respectively; p=0.02) and its uncitrullinated form REP-1 (6% and 2%, respectively; p<0.001). Positive antibodies against fib (negative control peptide for cit-fib) and CParg were also more common among those with PD compared to non-PD patients (26% and 3%; p<0.001, and 9% and 3%; p=0.06, respectively). After adjusting for confounders, patients with periodontitis had 43% (p=0.03), 71% (p=0.002) and 114% (p<0.001) higher CEP-1, REP1 and fib antibody titres, compared with patients without periodontitis. After stratification for smoking, non-smokers with PD, compared with non-PD, had significantly higher titres of antibodies against CEP-1 (103%, p<0.001), REP-1 (91%, p=0.001), vimentin (87%, p=0.002), and fib (124%, p<0.001), independent of confounders, confirming that the antibody response was not due to smoking. We have shown that PD is associated with an antibody response to both citrullinated and uncitrullinated peptides of the RA autoantigens, and primarily to the uncitrullinated variants. This loss of tolerance could then lead to epitope spreading to citrullinated epitopes as the autoimmune response in periodontitis evolves into that of pre-symptomatic RA (paper 4).

1.6.2. Lung disease in the induction of antibodies to citrullinated antigens

Given that citrullination, and anti-citrullinated protein antibody formation occurs in smokers, in his study we measured the autoantibody reactivity in a cohort of patients with lung disease (paper 5). We compared the autoantibody reactivity in subjects with usual chronic obstructive pulmonary disease (COPD) due to smoking (n=113), subjects with lung involvement due to α1-antitrypsin deficiency (AATD) (non-smokers, n=257), and healthy non-smokers (n=22). We measured ACPA (i.e. anti-cyclic citrullinated peptide (anti-CCP) and anti-mutated citrullinated vimentin (anti-MCV) antibodies) and also anti-elastin, given
that liberation of elastin peptides from the damaged lung may be a mechanism of autoimmune lung disease. Anti-elastin antibodies were higher in controls relative to AATD (p=0.008) and COPD (p<0.001), and in AATD relative to usual COPD (p<0.001). Anti-elastin levels showed a threshold at 10 pack-yrs, being higher in those who had smoked less (p=0.004). Smoking was the main determinant of anti-elastin antibody levels, which fall after 10 pack-yrs. With regards to ACPA, anti-CCP antibodies were higher in COPD than AATD (p=0.002) and were positive 8/257 (3.1%) AATD subjects and 6/113 (5.3%) of those with usual COPD. Anti-MCV antibodies were also higher in COPD than AATD and were positive in 34/257 (13.2%) of AATD subjects and 20/113 (17.7%) with usual COPD. These results suggests that the lung may contribute to the development of autoimmunity related to RA, by inducing antibodies to citrullinated autoantigens (paper 5)\textsuperscript{5}.

Taken together, the results of the two herein presented studies (papers 4 and 5 respectively), conducted among patients with other diseases that may predispose to the development of RA, i.e. periodontitis and lung disease, suggest that a breach of tolerance to citrullinated and uncitrullinated autoantigens may occur in association with disease at mucosal barriers, particularly in periodontitis. It is therefore conceivable that the pathogenesis of RA starts in tissues other than the joints, i.e., systemic autoimmunity is triggered by processes distant from the joints, a process which may or may not be followed by a ‘second hit’ leading to clinically apparent inflammatory arthritis in susceptible individuals. Individuals within the preclinical phase may be suitable targets in future strategies of primary prevention of RA. The spectrum of preclinical RA is wide, varying from individuals manifesting only with autoantibody production and no signs or symptoms to those who become symptomatic and then have clinically apparent arthritis.
1.7. Gender and other factors

Sex hormones or sex specific factors influence RA susceptibility. The majority (70%) of people with RA are women. Female hormones play a role in RA, possibly due to the stimulatory effects of oestrogen on the immune system. Various reproductive factors may be involved, as pregnancy is often associated with remission in the last trimester, with common postpartum disease flares, and also illustrated by the increased risk of RA associated with nulliparity, and RA risk reduction by breastfeeding for 1 year or more. Of note, men with RA have lower levels of testosterone and dehydroepiandrosterone (DHEA) and higher estradiol levels, although whether this is a consequence of inflammation is unknown. Stressful events such as emotional or physical trauma and a lower socioeconomic status have been related to the development of RA, while alcohol intake may be protective. The role of vitamin D as modifier of RA risk remains equivocal.

2. Synovial inflammation

RA is considered as an immune-mediated syndrome with involvement of both innate and adaptive immune systems. Different cells of the immune system and a broad range of cytokines, which trigger and amplify inflammatory pathways and promote the activation of other cells, such as fibroblasts, chondrocytes and osteoclasts, participate in a complex chain of events leading to persistence of synovial inflammation and active joint destruction.

Leucocytes migrate and infiltrate the synovium producing synovial inflammation. Migration of leucocytes is facilitated by endothelial activation in synovial microvasculature, increased expression of adhesion molecules and chemokines. Microscopic changes include neoangiogenesis induced by cytokines, insufficient lymphangiogenesis, which limits cell exit, and fibroblast activation resulting in the generation of synovial inflammatory tissue. While T cells are increased in the synovial tissue, their functional role remains unclear. Broad
spectrum targeting T cells or T cells deletion therapies have shown no efficacy\textsuperscript{98}, which may suggest the need to refine the target to specific T cell subsets\textsuperscript{31}. Myeloid cells and plasmacytoid dendritic cells, which express cytokines, HLA-II molecules and co-stimulatory molecules involved in T cell activation and antigen presentation\textsuperscript{99,100}, are increased in the RA synovium. T cell co-stimulation with abatacept, a fusion protein containing CTLA4 and the Fc fragment of IgG1, is effective in RA. The role of 17 helper T cells (Th17), a cell subset producing IL-17, 21, 22 and TNF-α, has been implicated in RA\textsuperscript{101}. Th17 differentiation, promoted by IL-1β, 6 21 and 23 and other mediators, does inhibit regulatory T cells, shifting the balance towards inflammation. IL-17 synergizes with TNF-α to activate fibroblasts and chondrocytes and inhibit regulatory T cells\textsuperscript{102}. The involvement of CD20+ B cells is confirmed by the efficacy of rituximab in RA\textsuperscript{103}. B cells are localized in T cell-B-cells aggregates as ectopic lymphoid follicles supported by APRIL, BlyS and chemokines in the synovium\textsuperscript{104}. Plasma cells in the synovium and periarticular bone marrow are not targeted by rituximab, thus the role of B cells in RA may involve autoantigen presentation and cytokine production, in addition to autoantibody production\textsuperscript{31}. Other cells of the innate immune system migrate to the synovial membrane in response to colony-stimulating factors, including macrophages, natural killer cells and mast cells. Macrophages are key players in synovial inflammation, activated by toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that recognize molecular patterns associated with pathogens and endogenous ligands\textsuperscript{105}, and also by cytokines, immune complexes, T cell interactions, lipoprotein particles and liver X-receptor agonists\textsuperscript{106}. Neutrophils in the synovial fluid, produce prostaglandins, proteases and reactive-oxygen species\textsuperscript{107}. Activated mast cells\textsuperscript{108} and their products (e.g. vasoactive amines, cytokines, chemokines, and proteases)\textsuperscript{109} activate chondrocytes, synovial fibroblasts and macrophages that contribute to joint inflammation\textsuperscript{110}. 


2.1. Cytokines

The inflammatory cytokine network contributes to the pathogenesis of RA\textsuperscript{111,112}. TNF-\(\alpha\) is a potent cytokine that activates cytokine and chemokine expression, endothelial-cell adhesion molecules expression, regulatory T cells suppression, and promotes angiogenesis\textsuperscript{113}. IL-6 mediates systemic effects such as anaemia, acute-phase responses, cognitive dysfunction, and lipid alterations. Consequently, inhibition of these cytokines has emerged as an important therapeutic target. IL-1 family cytokines promote cell activation of endothelial cells, leukocytes, chondrocytes and osteoclasts\textsuperscript{111}. IL-1 blockade however has shown limited clinical response. Other pathways targeting cytokines are under way, including B-lymphocyte stimulator (BlyS), IL-17, a proliferation-inducing ligand (APRIL), granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF), and RANKL receptor activator of NF-\(\kappa\)B ligand. The development of specific small-molecule inhibitors may evolve with the understanding of the intracellular signalling molecules such that regulate cytokine receptor mediated functions. Fostamatinib blockade of spleen tyrosine kinase, which is implicated in B-cell and Fc receptors, is effective in some patients\textsuperscript{114,115}, while tofacitinib, a inhibitor of Janus kinase (JAK) pathways which mediate the function of cytokines, interferons and other mediators\textsuperscript{116,117}.

2.2. Tissue response

RA is characterized by synovial hyperplasia with expansion of the membrane lining where fibroblast-like synoviocytes (FLS) display anchorage independence, loss of contact inhibition and expression of cytokines, chemokines, adhesion molecules, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs)\textsuperscript{118}. These cells contribute to cartilage destruction and persistence of inflammation, sustaining T-cell and B-cell survival and adaptive immune organization\textsuperscript{119}. FLS have been shown to migrate and spread RA to
unaffected joints. The molecular basis promoting synovial hyperplasia is unknown, but possible explanations in addition to FLS proliferation, include apoptosis resistance mediated by different pathways including stress proteins expression, p53 mutations and synoviolin, a E3 ubiquitin ligase regulating the cell proliferation/apoptosis balance. Other potential factors include methylation and acetylation of cell-cycle regulatory genes and altered expression of microRNA in fibroblast-like synoviocytes. Cadherin-11, a surface molecule, appears to have a critical role in the synovial lining processes and migration of FLS leading to damage.

2.3. Cartilage destruction

FLS adhesion and invasion is facilitated by the loss of lubricin, a secreted glycoprotein that protects cartilage surface inhibiting synovial cell overgrowth. FLS produce MMPs and other enzymes that degrade the collagenous cartilage matrix in a process not entirely opposed by endogenous enzyme inhibitors such as TIMPS, resulting in biomechanical alterations. Chondrocytes regulating matrix formation undergo apoptosis in response to cytokines in the synovium, which results in cartilage destruction seen as joint space narrowing on x-rays.

2.4. Bone destruction

Bone erosion occurs early in the course of the disease affecting 50% of cases within the first year. The periosteal surface adjacent to articular cartilage is invaded by osteoclasts in response to synovial cytokines, in particular NF-kB ligand (RANKL) and macrophage colony-stimulating factor. Osteoclast differentiation and activation is promoted by cytokines such as IL-1, 6, 17 and TNF-a, the therapeutic blockade of which reduces erosion formation. Osteoclasts enzymes destroy mineralized cartilage and subchondral bone leading to resorption pits that are seen as bone erosions on radiographs. Erosions occur in sites with a mechanical predisposition such as the second and third metacarpal bones. Bone marrow is accessed by synovial tissue through erosions resulting in bone marrow inflammation (seen as osteitis seen
on MRI), involving T-cell and B-cell aggregates\textsuperscript{133}. However, the temporal association in the interaction between the inflamed synovium and bone marrow in RA remains unknown. Whether the lesions on each side of the cortical bone occur simultaneously, or whether bone involvement (osteitis) precedes the erosion is unclear, thus RA may start in the bone marrow and then spread to the synovial tissue\textsuperscript{31}. However, bone repair of the erosions is unlikely. Chondroblast and osteoblast precursors differentiation from mesenchymal cells is inhibited by dickkopf-1 and frizzled-related protein-1 induced by cytokines\textsuperscript{134}. Understanding of the synovium-derived mesenchymal stem cells biology may open therapeutic strategies\textsuperscript{135,136}. 
E. Development of rheumatoid arthritis

The development of RA from preclinical to full-blown clinical disease can be understood using a metaphor of the growth of a plant from its seed to a mature plant as suggested by Hazes & Luime\textsuperscript{137}. There are no signs or symptoms of the disease in the preclinical phase, yet the genetic risk factors for RA are present, and the exposure to environmental risk factors, such as smoking, lung disease or periodontal disease, among others, may or may not have occurred. At this point, autoimmunity may develop, as suggested by the presence of autoantibodies such as ACPA or RF. This pre-articular phase can develop into a phase where joint pain is present without clinically objective synovitis. In the initial clinical phase, unspecific inflammatory arthritis can be identified but it is not classifiable. The disease develops in the next clinical phases to become fully classifiable into RA. The development of RA is illustrated on Figure 1. The spectrum of preclinical RA is wide, varying from patients manifesting only with autoantibody production and no signs or symptoms to those who have become symptomatic and have clinical synovitis. Recently a European League against Rheumatism (EULAR) recommendation for terminology to be used to define specific preclinical phases up to the development of RA has been published\textsuperscript{87}. Individuals would be described as having: (a) genetic risk factors, (b) environmental risk factors, (c) systemic autoimmunity associated with RA, (d) symptoms without clinical arthritis, (e) unclassified arthritis, and (f) RA, in the context of prospective studies. According to these recommendations, (a) to (e) can be used in any combination, for example, an individual may have (a) + (b), or (a) + (b) + (c), or (a) + (b) + (d), etc. Recognized biomarkers that are known to be present before the development of RA include for example presence of synovitis on imaging but not clinically, or presence of RF and/or ACPA, reflecting systemic autoimmunity.
associated with RA. People in the preclinical phase should be targeted in prevention strategies as early immune modulation may prevent disease among those at risk of RA\textsuperscript{26,27}.

1. Does time matter?

Early diagnosis of RA is essential to then implement an early management plan that may change the disease course to improve patient outcomes. Effective treatment strategies became available in the 1990s and it is now widely accepted that these strategies should be implemented early in the disease course\textsuperscript{138}. Compared with delayed treatment, early initiation of disease-modifying drugs (DMARDs) in recent-onset RA is more effective in inducing disease-remission\textsuperscript{139-141}, preventing progression of structural damage\textsuperscript{139,142,143}, and preserving physical function and work ability\textsuperscript{141,144,145}. Identifying people at risk for RA, within the spectrum of preclinical disease, is important as these should be targeted in future strategies for secondary prevention of RA.

2. Is there a therapeutic window of opportunity?

It is now clear that outcome is improved by the early introduction of disease modifying anti-rheumatic drugs (DMARDs)\textsuperscript{6}. We have shown in a meta-analysis of observational studies and randomized controlled trials that there is a critical period during which antirheumatic therapy should be initiated\textsuperscript{6}, a therapeutic window of opportunity early in the course of RA, associated with sustained benefit on structural damage (paper 6)\textsuperscript{6}. Early identification of patients with RA is essential to allow the prompt institution of therapy. However, the timely initiation of antirheumatic therapy requires an early classification or diagnosis of RA, or of early arthritis at risk of developing into persistent and erosive disease.
F. Prediction models for the development of rheumatoid arthritis

Prevention of structural damage is an important goal of therapy and early combination therapy improves outcomes in RA; thus, it is important to improve the prediction of RA early in the disease course. Clinical prediction rules have been developed to improve the early identification of patients at risk for RA before the 2010 ACR/EULAR classification criteria for RA. The most widely used models were developed using data from the Leiden early arthritis clinic. The first model was developed to predict persistent and persistent erosive disease in all patients with early arthritis. This model, also known as the Visser score, includes information on weighted variables, which are symptom duration, morning stiffness duration, arthritis in 3 or more joints, MTP tenderness, RF positivity, ACPA positivity, and erosions. The AUC of the Visser score was 0.84 for discriminating between self-limiting and persistence, and 0.91 for discriminating between erosive and non-erosive persistence. A second model was developed by the same group to predict the development of undifferentiated arthritis into RA as defined by the ACR 1987 criteria. This model, also known as the Leiden score, included other weighted variables, such as age, gender, global health, morning stiffness, pattern of joint involvement distribution, tender and swollen joint counts, RF and ACPA positivity, and CRP levels. The AUC for the Leiden model was 0.89 (SD±0.014). The Leiden score has been validated in the Birmingham cohort of newly presenting patients with very early synovitis. However, the prediction models are not perfect as they do not capture patients early in the disease course or with subclinical disease.

1. Role of ultrasound as a predictor of outcome in very early arthritis

Given that ultrasound imaging may detect joint inflammation and structural changes early in the disease course, in particular in the pre-articular phase when the presence of inflammatory arthritis is not observed by the physician, we investigated the role musculoskeletal ultrasound
as a predictor of outcome in people with arthritis at the very early stages of the disease course (paper 7). In this longitudinal study of newly presenting patients with very early synovitis, ultrasound demonstrated subclinical joint involvement in patients developing RA. Compared to the Leiden score, ultrasound significantly increased the AUC for predicting RA (AUC 0.905 vs. 0.962, respectively), indicating that ultrasound counts provide independently predictive data over and above the Leiden score. In particular, ultrasound scanning of the MCP, wrists and MTP joints provides the optimum minimal ultrasound data to improve RA prediction.
G. Classification criteria for rheumatoid arthritis

The classification criteria for RA were originally published in 1958 and revised in 1987\textsuperscript{23}. Evidence indicates that early diagnosis leading to early intervention is a strategy that reduces and even prevents RA consequences. Thus, prompt initiation of disease-modifying antirheumatic drugs (DMARDs) aiming at remission is the goal of therapy. The classification criteria do not perform well identifying early RA among cases of recent-onset arthritis\textsuperscript{150}. In 2010, the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) revised the criteria for identification of patients with newly presenting inflammatory arthritis who are at high risk of persistence and erosive damage, and for use as a basis for initiating disease-modifying therapy\textsuperscript{24}. Based on the data from existing early arthritis cohorts and previous prediction models\textsuperscript{146-148}, the new criteria include disease duration, the presence of RF or ACPA, the extent and pattern of joint involvement, and the presence of an acute phase response. However, the performance of these new criteria had not been tested in an independent cohort of patients with very early arthritis.

1. Performance of the 2010 RA classification criteria in very early arthritis

We assessed the performance of these new criteria in patients with early synovitis, seen within 3 months of the onset of inflammatory arthritis, and followed for 18 months to determine outcomes and the cumulative fulfillment of 2010 and 1987 criteria and management (\textit{paper 8})\textsuperscript{8}. Among 265 patients included in the study, 60 had alternative diagnoses at baseline. Of the remaining 205 patients, 20% met both 1987 and 2010 criteria, 3% met only 1987 criteria and 22% met only the 2010 criteria at baseline. The 2010 criteria, when applied at baseline, detected more patients who eventually required disease-modifying antirheumatic drugs (DMARDs), especially methotrexate, within the first 18 months. However, more patients whose disease eventually resolved without ever requiring DMARD were classified at baseline
as RA according to the 2010 criteria than with the 1987 criteria. We concluded that the 2010 ACR/EULAR criteria allow more rapid identification of patients requiring methotrexate compared with the 1987 ACR criteria when applied at baseline. However, if these criteria are to be used in very early disease, over diagnosis is an important issue to consider (paper 8)8. Our results were in line with recent studies indicating that the 2010 ACR/EULAR criteria are more sensitive for the identification of patients early in the disease course151,152. Importantly, when we investigated the role musculoskeletal ultrasound as a predictor of outcome in people with arthritis at the very early stages of the disease course, we found that 83% of those who developed RA met the 2010 ACR/EULAR criteria at baseline. When we replaced the clinical joint examination variables by joint ultrasound variables using these criteria, then 93% of patients were classified as RA at baseline, showing that ultrasound scanning does improve the prediction and classification criteria for RA early in the disease course (paper 7)7.
H. Disease outcome

The course of RA is variable. About 15-20% of patients have intermittent disease with flares and a relatively good prognosis. However, the majority have progressive disease with either a slow or a severe course. The outcome of RA depends upon the degree of structural damage, the physical function status, psychological health, and the presence of coexisting conditions.

1. Physical function

Functional capacity is a useful tool for assessing clinical effectiveness of therapeutic interventions. Functional disability indices are based on self-report questionnaires, which have been validated such as the Stanford Health Assessment Questionnaire (HAQ)\textsuperscript{153,154} and the Arthritis Impact Measurement Scale Health Status Questionnaire (AIMS)\textsuperscript{155}. However, these instruments focus on the physical impact, rather than the psychological impact of RA, which may contribute up to 20% towards disability\textsuperscript{156,157}. The SF-36, a generic health instrument consisting of 36 items organized in 8 domains including function and impact of physical disability and participation, is designed to assess overall health status\textsuperscript{158}.

2. Disease activity

Disease activity indicators include swollen and tender joint counts, pain, patient and evaluator global assessments of disease activity assessed (PGA and EGA, respectively), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), duration of morning stiffness, and fatigue. The disease activity scores (DAS) provides a global summative and continuous score for disease activity assessment\textsuperscript{159}. A simplification of the DAS, the DAS28, reduced the joints evaluated to 28 joints, is more practical and widely used in clinical trials and clinical practice\textsuperscript{160}. The DAS28 includes swollen and tender joint counts of 28 joints, global health assessment, erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP).
Ranges of disease activity based on DAS28 scores have been proposed: remission (DAS28<2.6), low (DAS28 2.6-3.2), moderate (DAS28 3.2-5.1), and high (DAS28>5.1). The Simplified Disease Activity Index (SDAI) uses five of the core set variables (SDAI = TJC28 + SJC28 + PGA + EGA + CRP) and gives a linear sum of untransformed variables\textsuperscript{161}. The SDAI has been validated and has high sensitivity and specificity for predicting change in DMARD therapy when compared to DAS28 at a cutpoint of 15\textsuperscript{162-164}. The Clinical Disease Activity Index (CDAI), a further simplification of the SDAI, uses the same measures as the SDAI but does not require CRP measurement (CDAI = TJC28 + SJC28 + PGA + EGA). The CDAI correlates well with other disease activity scores and response criteria, as well as disease progression and functional impairment\textsuperscript{161,165,166}.

Several self-reported instruments are available for the assessment of RA activity. These include the RA disease activity index (RADAI), the rapid assessment of disease activity in rheumatology (RADAR), and a modified Routine Assessment of Patient Index Data (RAPID3)\textsuperscript{167-170}. The RADAI has five items, including patient-assessed joint counts\textsuperscript{167} and the RADAR has six items relates to symptoms, function, work impact, psychological and social health and satisfaction\textsuperscript{168}. However, these instruments are rarely used in clinical trials. Patient centered outcomes most widely used are global assessments of disease activity with visual analog scales, HAQ scores and the SF-36. The RAPID3, expanded from the RAPID includes a HAQ, physical function, pain, patient global estimates, all normalized to 0-10, added and divided by 3 to give a score on a scale of 0-10\textsuperscript{169,170} and provides similar information to the DAS28 or the CDAI\textsuperscript{170}.
3. **Response criteria**

3.1. **ACR response criteria**

An early attempt to define minimal response were the Paulus criteria\textsuperscript{171}, which provided the basis for the ACR response criteria\textsuperscript{172}. The ACR criteria measure the frequency of benefit (i.e. the proportion of patients achieving a defined response) in a categorical response. The ACR20 response is defined as improvement of at least 20% in the number of both swollen and tender joint counts, as well as 20% improvement in $\geq3/5$ variables (PGA, EGA, pain visual analog scales, HAQ, acute phase response). The ACR50 and ACR70 criteria correspond to 50 and 70% improvement, respectively\textsuperscript{173}.

3.2. **EULAR response criteria**

The EULAR response criteria, based on the DAS28, categorize improvement into either good or moderate responses. A good response is defined by a decline in score $>1.2$ and results in the achievement of low disease activity (DAS28$<3.2$). A moderate response is defined by a decline in DAS28 by $>1.2$ or by a decline in DAS28 of 0.6 to 1.2 and reaching at least moderate disease activity (DAS28$<5.1$)\textsuperscript{174,175}. Disease activity varies in RA, partly due to endogenous rhythms of the disease but mainly as a result of therapeutic interventions, which depend on the efficacy, side effects or withdrawal of therapy. In contrast, structural damage is cumulative and irreversible. The degree of damage depends on disease activity and inflammation as well as repair\textsuperscript{176}. As structural damage progresses, disease activity assessment by clinical examination becomes more difficult. The symptom and signs of inflammation may be caused either by persistent disease or as a result of mechanical damage.
4. Remission criteria

Remission criteria defined by the American College of Rheumatology (ACR) are based on absence of symptoms for at least 2 consecutive months or low disease activity scores. However, clinical remission defined by these criteria does not rule out radiographic progression and disability, as clinical parameters do not appear to correlate with radiographic progression and structural damage. Therefore, because those in remission at one time need to have a low risk developing radiographic progression or loss of function, the ACR/EULAR has recently proposed new definitions of remission of RA\textsuperscript{177}, in which a number of different individual disease activity measures are considered at the same time. Based on these criteria, remission is achieved when the number of tender and swollen joints, the patient global assessment score (scale 0-10) and the CRP concentrations (mg/dl) are all ≤1. An alternative definition is a Simplified Disease Activity Index (SDAI) ≤3.3\textsuperscript{177}.
I. Disease progression and structural damage

Prevention of structural damage is an important goal of therapy. Radiographic imaging is considered the ‘gold standard’ for assessment of disease progression in RA\textsuperscript{178}, and used extensively in clinical trials as the primary outcome measure. Furthermore, radiographic assessment of joint damage is recommended by the Food & Drug Administration (FDA) as an outcome measure that supports the prevention of structural damage claims in clinical trials of pharmaceutical interventions in RA\textsuperscript{179}. The radiographic assessment using traditional scoring methods such as the Sharp or Larsen systems is subjective and based on a qualitative assessment of the joints\textsuperscript{180,181}. Other imaging modalities used in RA include ultrasound and magnetic resonance imaging (MRI), however, these are not widely adopted as they are operator dependant and/or expensive. A recent study observed that clinical and radiographic measures of RA generally correlated well with MRI. However, changes in MRI and clinical/radiographic measures did not correlate well, probably because MRI is more sensitive than radiographs and more objective than DAS28\textsuperscript{182}.

The available radiographic scoring methods do not attempt to provide a true measure of the size of the radiographic structures; rather, a score is given on an ordinal scale that is based on a comparison to representative examples. Several computerized techniques and software tools have been developed to measure radiographic changes on digitized radiographs. Computer-based methods to measure radiographic joint space width (JSW) have the potential to improve the longitudinal assessment of RA by providing an objective and continuous outcome measure with enhanced reliability and sensitivity to change.
1. Computer-based method to assess disease progression

We developed a semi-automated software application to measure radiographic JSW of the proximal interphalangeal (PIP), and metacarpophalangeal (MCP) joints on digitized hand radiographs. We assessed the diagnostic performance of a semi-automated computer-based method for measuring joint space width.

1.1. Sensitivity to change

We compared the diagnostic performance of a computer-based method for measuring JSW with the Sharp joint space narrowing (JSN) scoring method in a random sample of early RA patients with sequential hand radiographs scored with the Sharp method from the National Data Bank for Rheumatic Diseases. Hand joint space width was measured with our automated computer-based method in a blind and random order. We constructed a receiver operating characteristics curve and compared the diagnostic performance of the computer based and Sharp methods based on the areas under the curve. We included 129 early RA patients with radiographic follow-up. Changes in the software and Sharp methods were highly correlated ($R = 0.75$, $p<0.001$). The computer-based method was significantly more discriminant than the Sharp JSN subscale: The area under the curve of the software method was $0.96$ (95% CI 0.94, 0.99) compared to $0.93$ (95% CI 0.89, 0.96) for the Sharp subscale ($p = 0.024$). At the most discriminant cut-off, specificity of the software method was 88.4% (95% CI 81.5% - 93.3%) compared to 81.4% (95% CI 73.6% - 87.7%) for the Sharp subscale ($p = 0.04$); sensitivity was 87.6% (95% CI: 80.6% - 92.7%) for the computer-based method compared to 82.2% (95% CI: 74.4% - 88.3%) for Sharp subscale ($p = 0.13$). We observed that the software method for measuring JSW was more discriminant than the semiquantitative Sharp JSN subscale (paper 9). Our data suggests that image analysis software can be used to provide quantification of structural changes on a continuous scale (paper 9).
1.2. Repositioning reproducibility

An additional and previously unreported source of error is the change in JSW due to patient repositioning. In a subsequent study we measured the long-term patient repositioning reproducibility of software-measured radiographic JSW. We included 136 patients, selected from subjects in the National Data Bank for Rheumatic Diseases, with baseline and follow-up hand radiographs with a follow-up time of less than or equal to 3 years. To eliminate any JSW change due to real disease progression, evaluation was performed on unaffected joints, defined as having a total Sharp score of zero at both baseline and follow up. The Root Mean Square Standard Deviation (RMSSD) and coefficient of variation (CoV) were used as the reproducibility metrics. The RMSSD (CoV) was 0.14 mm (10.5 %) for all joints, 0.18 mm (10.9 %) for the MCP joints, and 0.08 mm (8.3 %) for the PIP joints. The distribution of JSW change was asymmetric suggesting that actually joint narrowing occurred for many of the joints. A second analysis was performed excluding joints where the loss of JSW was greater than three standard deviations. For this analysis, the RMSSD (CoV) was 0.10 mm (7.5 %) for all joints, 0.12 mm (7.2 %) for the MCP joints, and 0.07 mm (7.1 %) for the PIP joints. We observed that repositioning reproducibility is very good but is likely to be a dominating factor compared to reader and software reproducibility, and further evidence is given that a software method is able to detect changes in some joints for which the Sharp score is insensitive (paper 10)\textsuperscript{10}.

1.3. Reader reproducibility

The objective of this study (paper 11)\textsuperscript{11} was to evaluate the reproducibility of a computer-based method to measure the radiographic joint space width (JSW) in patients with RA. We used hand radiographs from a random sample of patients with RA. The semi-automated computer-based scoring system automatically delineated joint margins on MCP and PIP joints
to measure the JSW. Four readers independently evaluated digitized hand radiographs on two separate occasions. Inter-rater and intra-rater reproducibility was assessed with intraclass correlation coefficients (ICC), RMSSD and CV were used as the reproducibility metrics. The average reader time was 36 seconds per joint and 4.4 minutes per hand. Mean JSW was 1.65 mm and 1.03 mm for MCP and PIP joints, respectively. The inter-rater ICC values ranged between 0.96 and 0.98, and the RMSSD between 0.03 and 0.036. The CV was 2.2% and 2.9% for MCP and PIP joints, respectively. Regarding intra-rater reliability, the ICC ranged between 0.95 and 0.97, and the RMSSD between 0.02 and 0.031 mm. The CV was 1.8% and 2.2% for MCP and PIP joints, respectively. The proposed software-measured radiographic JSW is reliable, both in terms of interrater and intrarater reliability, and a short reader time. This semi-automated system has the potential for use in large clinical studies and should provide a quantitative, reproducible and more objective outcome measure of joint structural damage and response to therapy (paper 1111).
J. Are there systemic consequences to local disease?

Chronic inflammation in RA affects other organs such as the liver (anaemia of chronic disease and elevated acute phase response), lungs (inflammatory and fibrotic disease), brain (fatigue and cognitive function decline), muscles (sarcopenia), and bone (osteopenia, osteoporosis, fractures)\(^{31}\). Cytokines also make muscle and adipose tissues insulin-resistant, resulting in an inflammation-associated metabolic syndrome\(^{183,184}\). Inflammatory mediators, including cytokines, immune complexes, and altered lipid particles, circulate to promote several comorbidities in patients with RA\(^{31}\). The same inflammatory mediators involved in synovial pathology are also implicated in generating pathology in extra-articular tissues. Taken together, this suggests that chronic inflammation is the common theme linking the spectrum of RA-coexisting conditions (Figure 3).

Figure 3. Systemic consequences of rheumatoid arthritis
1. Cardiovascular disease in rheumatoid arthritis (RA)

Persons with RA have a reduced life expectancy and increased mortality compared with the general population. Epidemiologic studies have clearly demonstrated that an increase in prevalence of cardiovascular disease underlies the increased morbidity and mortality risk documented in patients with RA, and is independent of traditional cardiovascular risk factors or medications used to control the disease. RA is an independent risk factor for coronary artery disease. Patients with RA and systemic involvement have an increased risk of coronary events. The higher cardiovascular mortality rates in those with more severe disease support the notion that greater systemic inflammation confers additional risk. Preclinical atherosclerosis in RA is also associated with longer and more severe disease. Moreover, there is an increased cardiovascular disease (CVD) risk early in the course of RA, probably related to subclinical inflammation in the prearticular phase of RA. Inflammatory pathways that have been implicated include cytokines (e.g. IL-6 and TNF-α), acute-phase reactants (CRP and ESR), immune-complexes, and altered lipid particles (e.g. proinflammatory HDL) that increase endothelial activation and may contribute to plaque instability. An inflammation-associated metabolic syndrome has been suggested, which was associated with higher coronary calcification scores on computed tomography scan in patients with RA. Further, lipid biochemistry is closely linked to inflammation, thus active RA is associated with reduced serum levels of total, HDL, and LDL cholesterol, which may increase in response to therapy. Thus, inflammation plays a key role in atherogenesis associated with RA and its complications.

Importantly, other novel and potentially modifiable risk factors, such as antioxidant vitamins and serum uric acid levels, as well as other unknown CVD risk factors may account for the increased CVD risk in RA. Previous reports have shown inverse associations between
inflammation and antioxidant serum levels, in particular carotenoids\textsuperscript{198-200}. Moreover, intakes of antioxidant vitamins and other micronutrients or their serum concentrations, including carotenoids\textsuperscript{201-203}, vitamin C\textsuperscript{204-206}, vitamin E\textsuperscript{207,208}, and folate and homocysteine\textsuperscript{209-211} have been reported to be inversely associated with CVD incidence and mortality. An inverse association between vitamin D levels and CVD risk and risk factors has been suggested based on the biologic effects of vitamin D, which include immunomodulatory effects\textsuperscript{212,213}, antiproliferative effects on myocardiocytes\textsuperscript{214,215}, as well as effects on the renin-angiotensin system\textsuperscript{216,217}. Thus, in the context of a chronic inflammatory disease, deficiency of antioxidants and/or vitamins may be associated with accelerated atherosclerosis in RA.

\textbf{1.1. Antioxidants and other novel cardiovascular risk factors in RA}

We compared novel risk factors for CVD, including inflammatory biomarkers, antioxidants and vitamins, as well as traditional CVD risk factors in participants with RA and non-RA controls in a large population sample. RA was defined based on 1987 ACR criteria and non-RA subjects were defined as those who had no ACR criteria. We performed univariate and multivariate analyses of the association between RA and each novel and traditional CVD risk factor in RA vs. non-RA subjects. The sample included 5,302 subjects, 131 (2.5\%) with RA and 4,444 (84\%) without RA. Plasma levels of antioxidants $\alpha$-carotene, $\beta$-cryptoxanthin, lutein/zeaxanthin, and lycopene were significantly lower in RA compared with non-RA subjects in multivariate analysis adjusting for potential confounders. There was no difference in vitamin D levels between groups. Compared with non-RA participants, subjects with RA were more likely to have increased CRP levels in multivariate analysis adjusting for potential confounders. RA and non-RA participants had similar prevalence of traditional CVD risk factors and previous CVD. We concluded that the prevalence of traditional CVD risk factors in RA cases compared with controls was similar. Among novel CVD risk factors, C-reactive
protein (CRP) was significantly higher, and antioxidant levels were significantly lower in RA compared with non-RA (paper 12). CRP, a biomarker of inflammation, is an independent predictor of future vascular events in the general population. Antioxidant depletion can be seen in relation to inflammation; however, the lower antioxidant status in RA was not fully explained by the inflammatory process, suggesting that there might be other pathways involved in this association, which may be involved in the increased CVD risk in RA.

1.2. Serum uric acid and cardiovascular disease

Serum uric acid (SUA) is a potent endogenous antioxidant. Elevated serum uric acid (SUA) has been associated with cardiovascular disease (CVD) for decades. However, whether or not hyperuricemia has a direct, independent causal role in atherosclerosis has been debated. Some authors suggest that it is merely a marker of traditional risk factors for CVD, including the metabolic syndrome (Figure 4). However, there are other potential pathways for which experimental data exist that may implicate elevated SUA as a causal CVD risk factor, such as circulating platelet activation, low-density lipoprotein (LDL) oxidation, free radical release, vascular smooth muscle cell proliferation, crystal accumulation within atherosclerotic plaques, antiproliferative effects on endothelium and impaired endothelial nitric oxide production leading to endothelial dysfunction. Another possible mechanism is that uric acid may cause inflammation. Indeed, several in-vitro and experimental data support such a direct, proinflammatory role of SUA (Figure 4).

Inflammatory processes are involved in atherogenesis as well as in the rupture or erosion vulnerability of an atherosclerotic lesion. CRP is an independent predictor of future vascular events in the general population. Guidelines suggest measuring CRP as an aid to coronary risk assessment in adults without CVD.
1.2.1. Association between SUA and inflammation

Previous studies have suggested that SUA may have a direct role in atherogenesis; however, data from clinical and epidemiologic studies on such an association are scarce\textsuperscript{238-241}, particularly on a relationship between SUA and biomarkers of inflammation. Therefore we set out to evaluate the association of SUA with CRP and its dose-response relationship in a large representative sample of the US general population (paper 13)\textsuperscript{13}. The study sample included 10,882 participants. Participants taking gout medications or with hypouricemia were excluded. There were 4,942 men and 5,434 women. Men had mean higher SUA and lower CRP concentrations compared with women (SUA 6.01±1.3 vs. 4.71±1.3 mg/dl and CRP 0.38±0.88 vs. 0.55 ± 0.86 mg/dl, respectively). There was a positive linear dose-dependent association between SUA and CRP concentrations in both genders.
The associations were strongest in participants with BMI<25 and smallest in obese individuals (p for interaction: p<0.0001). There was no effect modification by age, race/ethnicity, or smoking. We have shown in this large epidemiologic study, an independent direct association between SUA and CRP in the general population. Even within the normal range and among those without comorbidities or taking prescription medications or NSAIDS/analgesics, serum uric acid concentrations were independently associated with higher CRP levels among men and women, particularly in lean and overweight individuals. Hyperuricemia associated with higher CRP concentrations. There was a positive linear association between SUA and CRP across the normal range of SUA concentrations. These associations were independent of comorbidities, medications and other potential confounders, and were modified by BMI. The results of our study confirm previous observations and show for the first time positive linear association between CRP and SUA, even across the normal range of SUA (Figure 4), which was independent of age, gender, race/ethnicity, income, smoking, alcohol intake, hypertension, GFR, BMI, physical activity, diabetes, arthritis, previous CVD, CHF, stroke, COPD, metabolic syndrome, insulin resistance, and prescription medications, with robust associations after excluding participants taking prescriptions medications or having comorbidities. These findings could have important clinical and public health implications as SUA may contribute to the atherosclerotic process by increasing inflammation.

1.2.2. Association between SUA and inflammation in RA

We have shown in a large epidemiologic study an independent association between SUA and CRP in the general population (paper 13). However, it is unclear whether this association holds in the context of a “high-grade” chronic inflammatory disease, such as RA. The purpose of this study was to evaluate the association between SUA and CRP in a cohort
of patients with RA (paper 14)\textsuperscript{14}. We included 400 consecutive patients with RA meeting ACR criteria for RA classification, recruited from routine outpatient clinics at the Department of Rheumatology of the Dudley Group of Hospitals. A priori we excluded participants with concomitant gout. We used fractional polynomial regression to evaluate the association between SUA and CRP, adjusting for age, gender, smoking, seropositivity, hypertension, diabetes, creatinine levels, glomerular filtration rate (GFR<60), body mass index (BMI), and use of medications such as diuretics, antihypertensives, aspirin, and statins. Models with further adjustment for presence of the metabolic syndrome, disease activity (DAS28), and medications used for RA (i.e. DMARDS, biologics, steroids) were also fitted. After excluding participants with concomitant gout, the study sample included 381 participants with RA. Of those, 74% were female, 76% were seropositive, 70% were hypertensive and 10% had diabetes. Mean age was 61 years (SD±12) and mean disease duration was 12.5 years (SD±10.5). Men had higher SUA and CRP concentrations than women (6.0±1.3 vs. 4.9±1.5 mg/dl and 17.3±20.8 vs. 16.8±23.5 mg/dl, respectively). There was a non-linear association between SUA and CRP concentrations (overall p-value 0.01) independent of confounders. We have shown that SUA concentrations are independently associated with inflammation among individuals with RA (Figure 4). The physiologic basis of this association, as well as its significance, both in terms of the articular and cardiovascular phenotype of RA require further exploration. However, the recognition RA as a risk factor for cardiovascular CVD is essential for clinicians, as important modifiable risk factors frequently remain untreated\textsuperscript{242}. Cardiovascular risk should be assessed and modifiable risk factors should be treated effectively in patients with RA. Furthermore, since chronic inflammation may be a modifiable risk factor for atherosclerosis, RA disease activity should be managed aggressively.
2. Bone loss in rheumatoid arthritis

The inflammatory process has adverse effects on bone remodeling, both focal and systemic\textsuperscript{243}. The impact of RA on bone includes structural joint damage (marginal erosions, subchondral bone erosions) and periarticular osteoporosis as well as generalized osteoporosis, which is multifactorial, including osteoporosis risk factors in addition to inactivity, inflammation, and use of corticosteroids. Bone formation is decreased in patients with RA not treated with steroids\textsuperscript{244}. Osteoporosis is frequent among patients with RA\textsuperscript{245-250}, and an increased risk of osteoporotic fractures across all age groups, gender and different anatomic sites has been shown\textsuperscript{251}. Vertebral fractures are common, with a prevalence of 15\% even after early DAS-steered treatment in RA patients\textsuperscript{252}. Bone loss in RA is associated with an imbalance in bone remodeling; the bone resorption process is mediated by osteoclasts via the RANKL–RANK–osteoprotegerin system\textsuperscript{253}. Cytokines, such as TNF-\textalpha, IL-1 and IL-17, accelerate the process of osteoclast differentiation by upregulating RANKL\textsuperscript{254}. Higher serum levels of CRP have also been associated with lower BMD in women and older adults. While chronic inflammatory diseases predispose to fractures and bone loss\textsuperscript{251,255}, it is unclear whether inflammation also has such an effect among people without chronic inflammatory diseases or whether this association holds in a representative sample of the general population.

2.1. Relationship between bone loss and inflammation

The purpose of this study was to examine the relationship between bone mineral density (BMD) and inflammation as measured by C-reactive protein (CRP) in a large representative US population-based sample from the National Health and Nutrition Examination Survey (NHANES). We included participants aged 20+ with BMD (total and subregions) measured by dual-energy x-ray absorptiometry (DXA) scans. The association between CRP and BMD was evaluated using multivariable linear regression models, adjusting for potential
confounders and further adjustment for comorbid diseases, medications and serum vitamin D levels. The study sample included 10,475 participants, among which there were 53% Caucasian, 22% Mexican-American, 18% African-American, and 7% were other races. Men had higher BMD and lower CRP concentrations than women. BMD (total, subtotal, extremities, ribs, and trunk subregions) was inversely associated with CRP quintiles both in men and women in a dose-dependent manner (total BMD p for trend: <0.0001 for men; 0.0005 for women). The associations were independent of medications, comorbidities and other potential confounders. The results remained largely unchanged with further adjustment for serum vitamin D levels. We concluded that among men and women in a large representative population-based sample, CRP was inversely and independently associated with total BMD in a dose-dependent manner\textsuperscript{15}(paper 15). We then confirmed these results in a European population-based sample\textsuperscript{256}. These data support the notion that, even among individuals without chronic inflammatory diseases, inflammation has a detrimental impact on bone.
K. Management of rheumatoid arthritis

Over the last 15 years the management of RA has markedly expanded. Disease modifying drugs (DMARDs), of which methotrexate in particular, have replaced NSAIDS as first-line treatment\textsuperscript{257-259}. Increasing knowledge of RA pathogenesis has resulted in the development of new targeted treatments (Figure 5). The recently developed targeted treatments designed using a biotechnological approach, known as ‘biologics’, have supplemented and in part replaced conventional DMARDs. The first biologics registered for RA block TNFα, a cytokine which is present in the RA synovial tissue\textsuperscript{260}. The TNF blockers include infliximab (chimeric antibody), etanercept (a soluble receptor), adalimumab (a humanized antibody). More recently, certolizumab (a pegylated antibody fragment), and golimumab (a human monoclonal antibody) have been added to the anti-TNF armamentarium. Combination treatment of a TNF blocker and methotrexate, in patients who fail initial treatment with methotrexate or other DMARDs, is effective in a subset of patients. Other biologics registered for RA treatment are rituximab, a chimeric antibody which depletes CD20 positive cells, abatacept, which blocks the interaction between CD80 or CD86 on T cells and antigen presenting cells, and tocilizumab, which blocks the IL-6 receptor. These drugs decrease disease activity in a degree similar to that of TNF blockers.

Despite the armamentarium of drugs developed over the last few years, early disease remission is only achieved in a proportion of patients, and often patients have to be treated with relatively expensive treatments. Therefore, there is a need to better understand RA pathogenesis to further improve response to treatment.

Recent advances in drug development that show promise in RA management involve tofacitinib, a Janus-associated kinase (JAK) inhibitor. Tofacitinib binds and inhibits important intracellular enzymes JAK1, JAK2, and JAK3, which are involved in immune cell activation,
cytokine production and signalling. A phase 2B randomized controlled trial of tofacitinib compared with adalimumab, a TNF inhibitor, or placebo\textsuperscript{261}, showed that tofacitinib therapy resulted in a rapid clinical response compared with the other groups. Adverse events included urinary tract infections, anaemia and diarrhoea. Phase III studies on tofacitinib therapy are underway. Another tyrosine kinase inhibitor that targets spleen tyrosine kinase (Syk) has shown efficacy in RA\textsuperscript{115}.

Figure 5 summarizes the management pathway in RA. Once the patient has been classified\textsuperscript{24}, the antibody status and in particular the ACPA status should be defined. There are currently several therapeutic options in RA, which can be used with a treat-to-target strategy\textsuperscript{262}, with the ultimate goal of inducing clinical remission\textsuperscript{177}.

**Figure 5. Rheumatoid arthritis management pathway**
L. Summary

This thesis set out to provide an overview of RA within the epidemiology of musculoskeletal involvement, with a focus on the comorbidities and potential risk factors for RA, the relevance of time in the management of RA, the tools used to assess disease prediction and progression, and the role of chronic inflammation as the common theme linking the spectrum of RA-coexisting conditions and consequences.

Paper 1\(^1\) summarized the relevance of time for the burden of RA. With regards to the pathogenesis of RA, we investigated potential risk factors and their associations with RA (papers 2-5). Paper 2\(^2\) presented a systematic review showing that the majority of clinical and epidemiologic studies suggest that patients with RA have an increased prevalence of periodontitis and tooth loss. However, the strength of the association remains unclear, and all available studies are cross-sectional, thus the temporality of the association cannot be ascertained. Several causal and non-causal pathways could explain the observed association. Emerging evidence suggests that periodontitis may have a direct causal role by initiating and sustaining the auto-immune inflammatory response underpinning RA. Additional non-causal pathways include genetic, environmental and behavioural exposures common to both conditions. Further causal pathways may emerge from pharmacological, physiological and behavioural exposures associated with RA, affecting periodontitis incidence and/or progression in individuals with RA. Paper 3\(^3\) demonstrated that having RA was associated with 4-fold increase in the odds of periodontitis, and a 3-fold increase in the odds of edentulism (complete tooth loss) controlling for age, gender, race/ethnicity, and smoking in a population-based sample. Following on these observations, Paper 4\(^4\) investigated the presence of systemic RA-related autoimmunity among individuals periodontal disease. We tested for ACPA given that these antibodies are highly specific for RA and epitope spreading
arises several years prior to the onset of clinical features. We found that after stratification for smoking, non-smokers with PD, compared with non-PD, had significantly higher titres of antibodies against CEP-1, REP-1, vimentin, and fibrinogen, independent of age and sex. In patients without RA, periodontitis was associated with higher titres of antibodies to both citrullinated and uncitrullinated peptides in non-smokers. This data suggests that periodontitis may contribute to the development of autoimmunity related to RA, by inducing antibodies to uncitrullinated variants of citrullinated autoantigens.

**Paper 5** investigated the presence of systemic autoimmunity among individuals potentially at risk of RA based on other environmental exposures. In this study, we compared the ACPA reactivity among patients with lung disease, either due to a genetic deficiency (i.e., α-1 antitrypsin deficiency, AATD) or to smoking (i.e., COPD), which is an established risk factor for RA, and healthy non-smokers. With regards to ACPA, anti-CCP antibodies were higher in COPD than AATD. Anti-MCV antibodies were also higher in COPD than AATD and were positive in about 13% and 18% of AATD and COPD subjects, respectively. These data suggest that the lung may also contribute to the development of autoimmunity related to RA, by inducing antibodies to citrullinated autoantigens. Recognized biomarkers that are known to be present before the development of RA include the presence of RF and/or ACPA, reflecting systemic autoimmunity associated with RA.

Taken together, the results of the two herein presented studies (papers 4 and 5 respectively), conducted among patients with other diseases that may predispose to the development of RA, i.e. periodontitis and lung disease, suggest that a breach of tolerance to citrullinated and uncitrullinated autoantigens may occur in association with disease at mucosal barriers, particularly in periodontitis. It is therefore conceivable that the pathogenesis of RA starts in tissues other than the joints, i.e., systemic autoimmunity is triggered by processes distant from
the joints, a process which may or may not be followed by a ‘second hit’ leading to clinically apparent inflammatory arthritis in susceptible individuals. Individuals within the preclinical phase may be suitable targets in future strategies of primary prevention of RA. The spectrum of preclinical RA is wide, varying from individuals manifesting only with autoantibody production and no signs or symptoms to those who become symptomatic and then have clinically apparent arthritis. This is important as Paper 6 demonstrated in a meta-analysis of observational studies and randomized controlled trials, that there is a therapeutic window of opportunity early in the course of RA, a critical period during which antirheumatic therapy should be initiated, associated with sustained benefit on disease progression and structural damage. However, the timely initiation of anti-rheumatic therapy requires the early identification of patients at very high risk of RA or of early arthritis at risk of developing into persistent and erosive disease. To help identify individuals early in the course of RA, Paper 7 investigated the role of musculoskeletal ultrasound as a predictor of outcome in people with arthritis at the very early stages of the disease course and showed that ultrasound identifies subclinical joint involvement in patients developing RA and significantly improves the prediction of RA, over and above the Leiden score. Paper 8 examined the performance of the recent 2010 ACR/EULAR criteria for classification of RA in newly presenting patients with very early synovitis and observed that, compared with the 1987 ACR criteria, the new criteria allow more rapid identification of patients requiring methotrexate and are more sensitive for the identification of patients early in the disease course.

The goal of therapy in RA is the prevention of radiologically evident joint destruction or functional loss. The next few papers investigated the diagnostic performance of an automated computer-based scoring method to assess structural damage and disease progression in RA (papers 9-11). Paper 9 demonstrated that compared with the semiquantitative Sharp JSN
subscale, the computer-based method for measuring joint space width was more discriminant and responsive to change. In addition, it provides quantification of structural changes on a continuous scale. **Paper 10** showed that the long-term patient repositioning reproducibility is very good and that the software method is able to detect changes in some joints for which the Sharp score is insensitive. **Paper 11** demonstrated that the software method is reliable, both in terms of interrater and intrarater reliability, and requires a short reader time. Therefore, this semi-automated system has the potential for use in large clinical studies and should provide a quantitative, reproducible and more sensitive and objective outcome measure of joint structural damage and response to therapy.

The following papers addressed some of the systemic consequences of chronic inflammation, including cardiovascular disease in RA. **Paper 12** demonstrated that people with RA have a similar prevalence of traditional CVD risk factors and previous CVD than people without RA. Among the novel CVD risk factors, C-reactive protein was significantly higher, and antioxidant levels, including plasma levels of α-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene, were significantly lower in RA compared with non-RA. There was no difference in vitamin D levels between groups. CRP, a biomarker of inflammation, is an independent predictor of future vascular events in the general population. Antioxidant depletion can be seen in relation to inflammation; however, the lower antioxidant status in RA was not fully explained by the inflammatory process, suggesting that there might be other pathways involved in this association, which may be involved in the increased CVD risk in RA. Substantial data implicates serum uric acid (SUA) in cardiovascular morbidity. However, whether or not hyperuricemia has a direct, independent causal role in atherosclerosis has been debated. SUA may be just a marker of traditional risk factors for CVD, including the metabolic syndrome. However, there are other potential pathways between SUA and CVD.
including inflammation. Indeed, several in-vitro and experimental data support such a direct, proinflammatory role of SUA. However, whether this relationship exists in the general population is unclear. We demonstrated in Paper 13\textsuperscript{13} an independent direct association between SUA and CRP. Even within the normal range and among those without comorbidities or medications, SUA was independently associated with higher CRP levels among men and women, particularly in lean and overweight individuals. These findings could have important public health implications as SUA may contribute to the atherosclerotic process by increasing inflammation; however, whether this association holds in the context of a “high-grade” chronic inflammatory disease, such as RA is unclear. Paper 14\textsuperscript{14} demonstrated that SUA concentrations are independently associated with inflammation as measured with CRP among individuals with RA and may contribute to the increased CVD risk in RA. Paper 15\textsuperscript{15} illustrated in a large population-based sample that inflammation has a detrimental effect on bone mineral density even in people without chronic inflammatory diseases.

In summary, we have seen within the epidemiology of musculoskeletal involvement that chronic inflammation in any one tissue clearly impacts the overall health status and disease susceptibility of the whole body. Chronic inflammation has detrimental effects not only as a result of direct damage but also due to collateral damage in other systems and tissues. Inflammatory diseases in different compartments may interact at a systemic level to influence inflammatory burden, bone loss, periodontal disease and cardiovascular risk. Further exploration of the physiologic basis of this interaction, as well as its significance in terms of the phenotype of RA is warranted. In the meantime, a tight control of the inflammatory process aiming at remission should be the goal of therapy. Importantly, preventing the burden of RA may be achieved by identifying people not only very early in the disease course but also at risk of developing RA, to implement interventions before clinical symptoms start.
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Appendix

The enclosed papers encompass the work included in this thesis.
The full article text for Papers 1-3, 5-6, and 9-15 has been removed from the electronic version of this thesis due to copyright restrictions
Paper 1
Rheumatology
Does time matter in the management of rheumatoid arthritis?

Paola de Pablo, Andrew D Filer, Karim Raza and Christopher D Buckley

INTRODUCTION

Imagine that you wake up one morning and feel so much pain and stiffness in your joints that every movement is agony. You can no longer dress or wash yourself without help, let alone drive to work. Your general practitioner (GP) refers you urgently to a rheumatologist, who tells you that you have arthritis. Although very reassuring, your rheumatologist tells you that even with the results of blood tests, they do not yet know which type of arthritis you have. For the moment your doctors can offer only very general treatment until your arthritis changes to involve more joints in a particular pattern or until other tests such as radiographs show specific features, which may take months. Distressingly, your rheumatologist cannot tell you whether the arthritis will get better on its own or will remain and change your life forever.

This is the scenario that greets new patients with an inflammatory arthritis such as rheumatoid arthritis (RA), who make up 1% of our population. The truth is that we are still very poor at predicting both diagnosis and severity of arthritis in patients who present to early arthritis clinics. Frustratingly, new data from studies of the treatment of RA in the early stages suggest that very early treatment has the real potential to completely switch off disease – that is, to induce disease remission. Studies also show that the longer the duration of disease, the more likely it is to persist, so how can we resolve the problem that the best predictor of persistent disease in patients with early arthritis currently is time itself? Would we wait as long to intervene in patients with breast lumps or prostate abnormalities as we do in our patients with arthritis?

The last two decades have seen important advances in our understanding of the incidence, treatment and outcome of patients with early rheumatoid arthritis (ERA). It is now clear that patients with ERA benefit from early referral and assessment by a rheumatologist and that outcome is improved by the early introduction of disease-modifying antirheumatic drugs (DMARDs). With appropriate treatments, it is possible to delay or even prevent evolution of undifferentiated arthritis into RA and/or to induce remission in a substantial proportion of patients with RA. However, we are still far from being able to produce an individualised treatment plan for each patient, as is currently the case for patients with cancer. This is because our current understanding of RA probably embraces a number of disease subsets, each with a different aetiology and pathophysiology.
Paper 2
Periodontitis in systemic rheumatic diseases

Paola de Pablo, Iain L. C. Chapple, Christopher D. Buckley and Thomas Dietrich

Abstract | Periodontitis is a chronic inflammatory disease that is characterized by loss of the periodontal ligament and alveolar bone, and is a major cause of tooth loss. Results from clinical and epidemiologic studies have suggested that periodontitis and tooth loss are more prevalent in individuals with rheumatoid arthritis (RA). However, the strength and temporality of the association are uncertain. Several biologically plausible causal and noncausal mechanisms might account for this association between periodontitis and RA. There is evidence to suggest that periodontitis could indeed be a causal factor in the initiation and maintenance of the autoimmune inflammatory response that occurs in RA. If proven, chronic periodontitis might represent an important modifiable risk factor for RA. In addition, patients with RA might show an increased risk of developing periodontitis and tooth loss through various mechanisms. Moreover, exposure to common genetic, environmental or behavioral factors might contribute to a noncausal association between both conditions.

Introduction
Chronic periodontitis is arguably the most prevalent chronic inflammatory disease in humans. The defining feature of periodontitis is chronic inflammation of the tooth-supporting tissues, leading to the progressive destruction of periodontal ligament and alveolar bone. Periodontitis is a very common disease, affecting over 30% of adults aged 65 years or above in the UK.

Results from a growing number of clinical studies point towards a potential association between chronic periodontitis and systemic rheumatic diseases—in particular, rheumatoid arthritis (RA). If proven, such an association would be very important from a clinical and public health perspective for several reasons. First, there are several processes through which chronic periodontitis might be part of a causal pathway in the pathogenesis and/or disease activity status of RA. Given the high prevalence of chronic periodontitis, a large proportion of the incidence and/or morbidity of RA could be attributable to chronic periodontitis, if causality was confirmed. Importantly, chronic periodontitis would represent a modifiable risk factor, as very effective treatments for this disease are available. Second, chronic periodontitis would contribute to morbidity in patients with RA even if the association was noncausal. Periodontitis is a leading cause of tooth loss in adults, which can have important clinical consequences, including impaired nutritional status and quality of life. Furthermore, chronic periodontitis is associated with an increased incidence of coronary heart disease and stroke, and emerging evidence indicates that this association might, in part, be causal.

Chronic periodontitis
Chronic periodontitis is initiated by a pathogenic biofilm that accumulates around the gingival (gum) margin. Susceptible patients exhibit an abnormal immune-mediated inflammatory response to the constituent microflora, which destroys the periodontal attachment and supporting bone. The early inflammatory response to the biofilm is dominated by polymorphonuclear leukocytes (PMNLs), and data indicate that stimulation of pattern recognition receptors on these cells by periodontal bacteria and their products (for example, lipopolysaccharide) has a key initiating role in disease athenogenesis.

The gingival epithelium is also important in orchestrating the inflammatory response. Early in the disease process, this epithelium ulcerates to expose the underlying connective tissues and vasculature to the biofilm. The exposed surface area facilitates direct entry of biofilm organisms to the circulation during eating and tooth brushing. Peripheral blood PMNLs from patients with periodontitis seem hyper-reactive relative to controls with respect to the release of reactive oxygen species and PMNL-specific proteases. Levels of inflammatory markers are also elevated, and antioxidant defenses appear compromised. Importantly, traditional anti-infective periodontal therapies, such as scaling and root planing, with or without adjunctive antimicrobials, not only improve the symptoms of patients with periodontitis but also improve vascular endothelial dysfunction and antioxidant status and reduce PMNL hyper-reactivity.

There is also strong evidence for a genetic component to disease susceptibility in ~50% of patients, although this genetic basis remains poorly understood. In certain individuals, there is evidence of hyper-reactivity in PMNLs and monocytes as well as subtle variations...
Paper 3
Association of Periodontal Disease and Tooth Loss with Rheumatoid Arthritis in the US Population

PAOLA de PABLO, THOMAS DIETRICH, and TIMOTHY E. McALINDON

ABSTRACT. Objective. To test for an association of periodontitis and tooth loss with rheumatoid arthritis (RA).

Methods. The third National Health and Nutrition Examination Survey (NHANES III) is a nationally representative cross-sectional survey of noninstitutionalized civilians. We included participants aged ≥ 60 years who had undergone both musculoskeletal and dental examinations. RA was defined based on American College of Rheumatology criteria. Dental examinations quantified decayed and filled surfaces, missing teeth, and periodontitis. Periodontitis was defined as at least 1 site exhibiting both attachment loss and a probing depth of ≥ 4 mm. We classified dental health status as (1) no periodontitis, (2) periodontitis, or (3) edentulous (i.e., complete tooth loss). We performed multivariate multinomial logistic regression models with dental health status as the dependent and RA as the independent variables.

Results. The sample consisted of 4461 participants, of whom 103 were classified as having RA. Participants with RA had more missing teeth (20 vs 16 teeth; p < 0.001), but less decay (2% vs 4%; p < 0.001) than participants without RA. After adjusting for age, sex, race/ethnicity, and smoking, subjects with RA were more likely to be edentulous [odds ratio (OR) 2.27, 95% confidence interval (CI) 1.56–3.31] and have periodontitis (OR 1.82, 95% CI 1.04–3.20) compared with non-RA subjects. In participants with seropositive RA there was a stronger association with dental health status, in particular with edentulism (OR 4.5, 95% CI 1.2–17).

Conclusion. RA may be associated with tooth loss and periodontitis. (First Release Nov 15 2007; JRheumatol 2008;35:70–6)

Key Indexing Terms: RHEUMATOID ARTHRITIS PERIODONTAL DISEASE PERIODONTITIS TOOTH LOSS EDENTULISM DENTAL HEALTH

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial inflammation that results in destruction of joint tissues. Periodontitis is a chronic inflammatory disease characterized by loss of the periodontal ligament and alveolar bone, and is a major cause of tooth loss, particularly in the elderly. Tooth loss has important clinical consequences, including reduced dietary quality and quality of life.

Periodontitis and RA appear to share numerous characteristics including certain pathogenetic processes, cytokine profiles, markers of inflammation, association with HLA-DRB1, interleukin 1B (IL-1B) and tumor necrosis factor-α (TNF-α) polymorphisms, presence of citrullinated proteins and peptide epitopes, and rheumatoid factor (RF), presence of citrullinated peptides and antibodies. This suggests that subjects susceptible to RA may also have higher rates of periodontal disease.

Further, there are reasons to suspect that the role of periodontitis in RA might be based on more than just shared susceptibility. For example, induction of adjuvant arthritis in Lewis male rats is associated with periodontal breakdown, increased cytokines and matrix metalloproteinases in gingival tissues, and alveolar bone loss.

Studies have also demonstrated an antibody response against oral anaerobic bacteria in synovial tissue and serum, and the presence of oral bacterial DNA in the synovial fluid and serum of patients with RA. Also, periodontal pathogens may express the peptidyl arginine deiminase (PAD) enzyme responsible for citrullination of peptide antigens.

Indeed, several clinical studies suggest a possible association between periodontitis/tooth loss and RA, although some studies did not find a positive association. However, no population-based data on this association have been reported.

Our objective was to compare periodontal disease and tooth loss prevalence in subjects with and without RA in the US population.
EXTENDED REPORT

The autoantibody repertoire in periodontitis: a role in the induction of autoimmunity to citrullinated proteins in rheumatoid arthritis?

Paola de Pablo,1 Thomas Dietrich,2 Iain L C Chapple,3 Michael Milward,3 Muslima Chowdhury,4 Peter J Charles,4 Christopher D Buckley,1 Patrick J Venables4

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ABSTRACT

Background Studies suggest that periodontitis may be a risk factor for rheumatoid arthritis (RA). The purpose of this study was to determine whether periodontitis is associated with autoantibodies characteristic of RA.

Methods Serum samples were tested for anti-cyclic citrullinated peptide (CCP), anti-mutated citrullinated vimentin (MCV), anti-citrullinated α-enolase peptide-1 (CEP-1), anti-citrullinated vimentin (cit-vim), anticitrullinated fibrinogen (cit-fib) and their uncitrullinated forms anti-CParg (negative control for anti-CCP), anti-arginine-containing α-enolase peptide-1 (REP-1), anti-vimentin and anti-fibrinogen antibodies in patients with and without periodontitis, none of whom had RA.

Results Periodontitis, compared with non-periodontitis, was associated with a normal frequency of anti-CCP and anti-MCV (<1%) but a higher frequency of positive anti-CEP-1 (12% vs 3%; p=0.02) and its uncitrullinated form anti-REP-1 (6% vs 2%; p<0.001). Positive antibodies against uncitrullinated fibrinogen and CParg were also more common among those with periodontitis compared to non-periodontitis patients (26% vs 3%; p<0.001, and 9% vs 3%; p=0.06). After adjusting for confounders, patients with periodontitis had 43% (p=0.03), 71% (p=0.002) and 114% (p<0.001) higher titres, compared with non-periodontitis. Non-smokers with periodontitis, compared with non-periodontitis, had significantly higher titres of anti-CEP-1 (103%, p<0.001), anti-REP-1 (91%, p=0.001), anti-vimentin (87%, p=0.002), and anti-fibrinogen (124%, p<0.001), independent of confounders, confirming that the autoantibody response in periodontitis was not due to smoking.

Conclusions We have shown that the antibody response in periodontitis is predominantly directed to the uncitrullinated peptides of the RA autoantigens examined in this study. We propose that this loss of tolerance could then lead to epitope spreading to citrullinated epitopes as the autoimmune response in periodontitis evolves into that of presymptomatic RA.

BACKGROUND

Rheumatoid arthritis (RA) is a chronic immune-mediated inflammatory disease characterised by synovial inflammation and cartilage and bone destruction that often results in structural damage, disability and functional loss. Causes of RA remain unknown but a complex interplay between genetic and environmental factors is involved in its development.1 A key feature of RA is the presence of antibodies to citrullinated peptide/protein antigens (ACPA) and these have now been included as a criterion in the recent American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) RA classification criteria.2 ACPA can be detected with generic assays such as citrullinated peptide antibodies (anti-CCP)3 or antibodies to distinct autoantigens such as citrullinated fibrinogen, vimentin, α-enolase and collagen.4 ACPA levels are predictive of disease severity and adverse clinical outcomes,5 6 and increasing evidence implicates ACPA in RA aetopathogenesis.4

Studies have suggested an association between RA and periodontitis.7 8 Periodontitis, one of the most common chronic inflammatory diseases, is characterised by loss of the periodontal ligament and alveolar bone, and is a major cause of tooth loss. Periodontitis is initiated by a pathogenic biofilm accumulating above and below the gum margin, which in susceptible patients triggers a dysregulated inflammatory immune response that causes collateral host-tissue damage.9–11 Periodontal bacteria enter the circulation and trigger an acute-phase response involving C-reactive protein and fibrinogen,12 and may activate neutrophils.13 Porphyromonas gingivalis, a common periodontal pathogen associated with periodontitis, is the only prokaryote known to express an endogenous peptidyl-arginine deiminase (PPAD) enzyme14 that citrullinates human proteins,15 which are abundantly present in periodontal tissues.16 It is therefore conceivable that increased citrullination of human or bacterial proteins by PPAD, or host peptidyl-arginine deaminases (PAD), in patients with periodontitis may result in a break in immune tolerance to citrullinated proteins and play a causal role in the initiation of RA.

It has therefore been hypothesised that RA-specific autoimmunity may be generated within the periodontium and that periodontal citrullination may be important in generating autoantigens in periodontitis.17 Once systemic tolerance is lost, epitope spreading and cross-reactivity may result, perpetuating the immune response leading to RA. ACPA appear many years before disease onset,18–22 in the preclinical phases up to the development of RA,23 suggesting that the initial immune disregulation occurs years before symptom onset, although how this takes place initially is yet to be determined. Interestingly, in a recent study using an animal model, immunisation with both uncitrullinated and citrullinated enolase caused rapid-onset...
controls, but these studies have all been small and differences in the presence and/or titres of ACPA compared to enolase, indicating that the initiation of the response was not necessarily citrulline dependent.

Few studies have shown that patients with periodontitis have a slightly increased presence and/or titres of ACPA compared to controls, but these studies have all been small and differences between patients and controls were not statistically significant. Moreover, antibodies to peptides and their uncitrullinated controls from specific citrullinated antigens have not been investigated. The purpose of this study was to determine the immune reactivities to peptides from citrullinated and uncitrullinated antigens in non-RA individuals with and without periodontitis.

**METHODS**

**Study sample**

The study sample comprised patients who had been referred to Birmingham Dental Hospital for treatment of periodontitis or for surgical removal of third molars who had been enrolled in clinical trials on outcomes of periodontal treatment and lower third molar surgery, respectively. Patients in the periodontal study had moderate or advanced periodontitis and were otherwise healthy and did not meet any of the following exclusion criteria: pregnancy, lactation, course of anti-inflammatory therapy or vitamin supplementation within the previous 3 months, regular mouthwash use, and any special dietary requirements (e.g., coeliac disease). The presence of moderate to advanced periodontitis was confirmed by clinical and radiographic examination. Patients in the third molar study required surgical removal of at least one lower third molar with the following exclusion criteria: pregnancy, lactation, regular use of anti-inflammatory medication or vitamin supplementation. The presence or absence of periodontitis was ascertained by determination of bone loss from radiographs of the dentition. Smoking status was collected at the study visit and classified as never, former and current. All subjects provided written informed consent and studies were approved by the NHS Research Ethics Service (REC ref 05/Q2707/252 and South Birmingham LREC/0405).

**Antibody measurements**

Fasting blood samples were collected at baseline, that is, before initiation of any dental treatment and immediately processed, aliquotted and stored at −80°C until analysis.

Anti-CCP antibodies were quantified using second-generation assays (Phadia UniCAP system; Phadia Ltd, Milton Keynes, UK). Antibodies against mutated citrullinated vimentin (anti-MCV IgG) were determined using a quantitative immunometric enzyme immunoassay (ORGENTEC Diagnostika GmbH, Mainz, Germany). These are clinically validated tests with a cut-off for positivity of 10 U/ml and 20 U/ml, respectively.

Antibodies to the uncitrullinated equivalent of CCP (CParg) were measured by ELISA. Serum diluted 1:100 were incubated on plates pre-coated with the antigen (kindly provided by Eurodiagnostica, Malmo Sweden) for 30 min. Plates were washed and incubated with peroxidase-conjugated mouse anti-human IgG (Hybridoma Reagent Laboratory, Baltimore, USA) (diluted 1:3000 in RIA buffer) for 1 h at room temperature. After a final wash to remove unbound conjugate, bound antibodies were detected with TMB substrate (Biolegend, San Diego, USA). The reaction was stopped by the addition of 1 M H2SO4 and absorbance was measured at 450 nm. OD values obtained for each sample were evaluated against a panel of normal healthy individuals to determine relative reactivity.

Antibodies to immunodominant peptides from established RA autoantigens were also measured, including citrullinated α-enolase (amino acids 4–21: KIHA-Cit-EIFDS-Cit-GNPTVE) (CEP-1), citrullinated vimentin (amino acids 60–75: VYAT-Cit-SSAV-Cit-L-Cit-SSVP)21 and citrullinated fibrinogen β chain (amino acids 36–52: NEEGFSSA-Cit-GHRPLDDK). Antibody titres were standardised and expressed as percentage differences between patients with and without periodontitis.

Statistical analysis

Summary statistics were calculated as appropriate. For univariate comparisons between patients with and without periodontitis we used χ² and Mann–Whitney U tests for categorical and continuous variables, respectively. Multiple linear and logistic regression models were used to compare titres of antibodies to citrullinated and uncitrullinated antigens and prevalence of sero-positivity between patients with and without periodontitis, adjusting for age, sex and smoking. Antibody titres were log transformed to achieve normality for linear regression models. To facilitate interpretability of the resulting estimates, antibody titres were standardised and expressed as percentage differences between groups, calculated as (e^x – 1)×100. STATA statistical software was used for all analyses.

**RESULTS**

The total sample included 194 participants, of whom 96 had periodontitis and 98 did not have periodontitis, with a mean age of 46 years (SD=8.9) and 29 years (SD=7.3), respectively. The proportion of women was similar in both groups (62% and 59%, respectively). Current smoking was prevalent in 24% of subjects with periodontitis and 22% of the non-periodontitis subjects. Ever smoking, defined as current and previous smoking, was present among 29% and 43% of the patients with and without periodontitis (p=0.02), respectively. None of the participants had a diagnosis of RA at the time of the study.
Antibody seropositivity

There was a low prevalence of antibody seropositivity to the generic tests for ACPA including anti-CCP and anti-MCV with frequencies around 1% in both periodontitis and non-periodontitis subjects (figure 1). Among the antibodies to peptides from specific citrullinated antigens, anti-CEP-1 seropositivity was significantly higher in patients with periodontitis compared with non-periodontitis (12% and 3%, respectively; p=0.02). There was no significant difference in the frequency of anti-citrullinated vimentin (cit-vim) or anti-citrullinated fibrinogen (cit-fib) (range 2–5%) in periodontitis and non-periodontitis. The prevalence of antibody seropositivity to the non-citrullinated peptides REP-1 (arginine control peptide for CEP-1) and fibrinogen was much higher in periodontitis. Anti-REP-1 seropositivity was found in 16% with periodontitis compared with 2% in non-periodontitis (p<0.001). Similarly, anti-fibrinogen (negative control peptide for cit-fib) and CParg antibodies (negative control peptide for CCP) were also more common among those with periodontitis compared to non-periodontitis patients (26% vs 3%; p<0.001, and 9% vs 3%; p=0.06, respectively).

Logistic regression models

Logistic regression analyses showed that patients with periodontitis were significantly more likely to have positive anti-CEP-1 (OR 4.1, 95% CI 1.1 to 15) compared to patients without periodontitis, but this difference was attenuated after adjusting for age. After controlling for major confounders, patients with periodontitis were significantly more likely to be anti-REP-1 and anti-fibrinogen positive compared with patients without periodontitis (OR 7.11, 95% CI 1.4 to 36 and OR 10, 95% CI 2.5 to 40, respectively) (table 1).

**Antibody titres**

The distribution of the antibody repertoire in periodontitis compared with non-periodontitis status is shown in figure 2. Serum antibody titres were significantly higher in patients with periodontitis compared with those without periodontitis for antibodies against CEP-1 (p<0.0001), REP-1 (p<0.0001), cit-vim (p=0.003), vimentin (p<0.0001), and fibrinogen (p<0.0001), but not cit-fib (p=0.1) (figure 2). There was no difference between the two groups in anti-CCP, anti-CParg and anti-MCV levels.

**Linear regression models**

Univariate analyses confirmed that antibody titres were significantly higher in patients with periodontitis compared with those without periodontitis for antibodies against cit-vim, vimentin, CEP-1, REP-1 and fibrinogen, but not cit-fib. These differences between groups were attenuated by adjustment for age but remained strongly statistically significant (table 2).

Multivariate regression analyses showed that compared to those without periodontitis, patients with periodontitis had 43% (p=0.03), 71% (p=0.002) and 114% (p<0.0001) higher anti-CEP-1, anti-REP1 and anti-fibrinogen titres, after adjustment for age, sex and smoking (figure 3). There was no difference between groups in anti-CCP, anti-CParg, anti-MCV, anti-cit-vim and anti-vimentin titres in multivariate analyses.

There was a highly significant interaction between periodontitis and smoking for anti-CEP-1 (p<0.001) and anti-vimentin antibodies (p<0.001) in age and sex-adjusted models (table 3). Among non-smokers, patients with periodontitis had significantly higher titres of anti-CEP-1 (103%, p<0.001), anti-REP-1 (91%, p=0.001), anti-vimentin (87%, p=0.002), and anti-fibrinogen (124%, p<0.001), compared with non-periodontitis patients, independent of age and sex.

The levels of anti-cit-vim and anti-cit-fib were also higher among non-smokers with periodontitis compared to non-smokers without periodontitis, although the differences did not reach statistical significance in multivariate models (p=0.30 and p=0.15, respectively). Among smokers, periodontitis was associated with increased anti-fibrinogen antibodies (98%, p=0.006) independent of age and sex. No difference was observed for other antibodies (table 3).

The prevalence of seropositive antibody reactivity to citrullinated and non-citrullinated peptides by periodontitis and smoking status is shown in figure 4. Among smokers, those with......

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**Table 1  Logistic regression models**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Univariate</th>
<th>Age-adjusted</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>CEP-1</td>
<td>4.1 (1.1 to 15)</td>
<td>0.03</td>
<td>2.14 (0.5 to 8.9)</td>
</tr>
<tr>
<td>REP-1</td>
<td>8.9 (2.0 to 40)</td>
<td>0.004</td>
<td>6.6 (1.3 to 34)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>11.2 (3.2 to 38)</td>
<td>&lt;0.001</td>
<td>10.2 (2.5 to 41)</td>
</tr>
</tbody>
</table>

*Age, sex and smoking adjusted model.
Periodontitis had a higher frequency of positive anti-CParg (p=0.004) and anti-fibrinogen (p=0.007) compared to non-periodontitis. Among non-smokers, patients with periodontitis had a higher prevalence of positive anti-CEP1 (p=0.02), anti-REP1 (p=0.005) and anti-fibrinogen antibodies (p<0.001) than patients without periodontitis.

Figure 2  Antibody repertoire in patients with and without periodontal disease. The boxes designate the 25th and 75th centiles, while the line in the boxes indicating the median number. The error bars indicate the minimum and maximum values. The numbers on the x axis indicate the periodontal disease (periodontitis; PD) status. CEP-1, citrullinated α-enolase peptide-1 antibodies; fib, citrullinated fibrinogen antibodies; vim, citrullinated vimentin antibodies; fib, uncitrullinated fibrinogen antibodies; REP-1, arginine-containing α-enolase peptide-1 antibodies; vim, uncitrullinated vimentin antibodies.

Table 2  Linear regression analyses with antibody titres shown as percentage differences in patients with periodontal disease (periodontitis) compared with patients without periodontitis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Univariate</th>
<th>Periodontitis</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Δ</td>
<td>95% CI</td>
<td>p Value</td>
</tr>
<tr>
<td>Cit-vim</td>
<td>39</td>
<td>5 to 84</td>
<td>0.02</td>
</tr>
<tr>
<td>Vimentin</td>
<td>63</td>
<td>24 to 114</td>
<td>0.001</td>
</tr>
<tr>
<td>CEP-1</td>
<td>110</td>
<td>62 to 173</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>REP-1</td>
<td>111</td>
<td>63 to 175</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cit-fib</td>
<td>21</td>
<td>−9 to 60</td>
<td>0.19</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>128</td>
<td>76 to 194</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Antibody concentrations were log transformed for analyses.
*Adjusted for age, sex and smoking.
DISCUSSION

To our knowledge, this is the first study to examine the immune reactivity profile to citrullinated antigens and their uncitrullinated forms among individuals with and without periodontitis. The finding of an increased prevalence and titre of antibodies to citrullinated peptides and/or their uncitrullinated controls in periodontitis supports the hypothesis that periodontitis might induce the autoimmunity that evolves into that of established RA. One major unexpected finding was that antibodies to the uncitrullinated controls such as CParg and the fibrinogen peptide were considerably increased in periodontitis relative to their citrullinated variants, which in turn suggests that it may be the uncitrullinated variants of the peptides that break tolerance in periodontitis.

Of the ACPA measured in this study, only one, anti-CEP-1, showed a significantly increased frequency in periodontitis. A similar increase was seen with antibodies to REP-1, suggesting that the anti-CEP-1 response was not citrulline specific. The increased prevalence of autoantibodies to both peptides was confirmed by a highly significant increase in antibody titres in periodontitis. This may be due to crossreaction with the bacterial enolase from P. gingivalis, a common periodontal pathogen associated with periodontitis. Evidence that this crossreaction may be relevant in vivo was previously demonstrated in an animal model, in which immunisation of DR4 transgenic mice with P. gingivalis enolase, both citrullinated and uncitrullinated, caused arthritis and antibodies to CEP-1 and REP-1. Therefore the antibody response that we observed in periodontitis could be induced by bacterial enolases from P. gingivalis or other bacteria within the pathogenic biofilm.

One of the most striking findings from this study was the significantly increased frequency and levels of antibodies to the uncitrullinated fibrinogen peptide in periodontitis. This cannot

Table 3  Multivariate linear regression shown as percentage differences in antibody titres in periodontitis subjects compared with non-periodontitis subjects, stratified by smoking

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Smokers</th>
<th>95% CI</th>
<th>p Value</th>
<th>Non-smokers</th>
<th>95% CI</th>
<th>p Value</th>
<th>p For interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cit-vim</td>
<td>-21</td>
<td>-52 to 32</td>
<td>0.37</td>
<td>24</td>
<td>-18 to 87</td>
<td>0.30</td>
<td>0.13</td>
</tr>
<tr>
<td>Vimentin</td>
<td>-33</td>
<td>-53 to 9.3</td>
<td>0.11</td>
<td>87</td>
<td>26 to 176</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CEP-1</td>
<td>-22</td>
<td>-50 to 23</td>
<td>0.28</td>
<td>103</td>
<td>41 to 193</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>REP-1</td>
<td>41</td>
<td>-13 to 129</td>
<td>0.16</td>
<td>91</td>
<td>29 to 182</td>
<td>0.001</td>
<td>0.28</td>
</tr>
<tr>
<td>Cit-fib</td>
<td>-12</td>
<td>-48 to 65</td>
<td>0.49</td>
<td>37</td>
<td>-11 to 108</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>98</td>
<td>22 to 220</td>
<td>0.006</td>
<td>124</td>
<td>52 to 230</td>
<td>&lt;0.001</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Linear regression models adjusted for age and sex.

A similar increase was seen with antibodies to REP-1, suggesting that the anti-CEP-1 response was not citrulline specific. The increased prevalence of autoantibodies to both peptides was confirmed by a highly significant increase in antibody titres in periodontitis. This may be due to crossreaction with the bacterial enolase from P. gingivalis, a common periodontal pathogen associated with periodontitis. Evidence that this crossreaction may be relevant in vivo was previously demonstrated in an animal model, in which immunisation of DR4 transgenic mice with P. gingivalis enolase, both citrullinated and uncitrullinated, caused arthritis and antibodies to CEP-1 and REP-1. Therefore the antibody response that we observed in periodontitis could be induced by bacterial enolases from P. gingivalis or other bacteria within the pathogenic biofilm.

One of the most striking findings from this study was the significantly increased frequency and levels of antibodies to the uncitrullinated fibrinogen peptide in periodontitis. This cannot

Figure 3  Antibody titres shown as percentage differences in patients with periodontal disease (periodontitis) compared with patients without periodontitis. The dark blue columns represent the results of univariate linear regression models and the light blue columns show the results of multiple linear regression models controlling for age, sex and smoking. p Values: *p<0.001; **p=0.02; §p=0.002; §§p=0.03.

Table 4  Prevalence of positive citrullinated and uncitrullinated antibody reactivity by periodontal disease and smoking status. Among non-smokers, patients with periodontitis (PD) had a higher prevalence of positive anti-CEP-1 (*p=0.02), anti-REP-1 (*p=0.005), and anti-fibrinogen (*p<0.001) than patients without PD. Among smokers, those with PD had a higher frequency of positive anti-CParg (*p=0.004), and anti-fibrinogen (*p=0.007) compared to non-PD. CCP, citrullinated peptide antibodies; CEP-1, citrullinated α-enolase peptide-1 antibodies; cit-fib, citrullinated fibrinogen antibodies; cit-vim, citrullinated vimentin antibodies; fib, uncitrullinated fibrinogen antibodies; REP-1, arginine-containing α-enolase peptide-1 antibodies; vim, uncitrullinated vimentin antibodies.

Figure 4  Prevalence of positive citrullinated and uncitrullinated antibody reactivity by periodontal disease and smoking status. Among non-smokers, patients with periodontitis (PD) had a higher prevalence of positive anti-CEP-1 (*p=0.02), anti-REP-1 (*p=0.005), and anti-fibrinogen (*p<0.001) than patients without PD. Among smokers, those with PD had a higher frequency of positive anti-CParg (*p=0.004), and anti-fibrinogen (*p=0.007) compared to non-PD. CCP, citrullinated peptide antibodies; CEP-1, citrullinated α-enolase peptide-1 antibodies; cit-fib, citrullinated fibrinogen antibodies; cit-vim, citrullinated vimentin antibodies; CParg, antibodies to the uncitrullinated equivalent of CCP; fib, uncitrullinated fibrinogen antibodies; REP-1, arginine-containing α-enolase peptide-1 antibodies; vim, uncitrullinated vimentin antibodies.
be explained by molecular mimicry because, unlike enolase, there is no homologue of fibrinogen in prokaryotes. One explanation may be found in the extraordinary efficiency with which two enzymes from \textit{P. gingivalis}, arginine-glycine-proline and PPAD, can degrade human fibrinogen into small C-terminally citrullinated peptides.\textsuperscript{14} This reaction, which occurs in less than 1 min in culture, produces peptides that would not occur with human PAD because human PAD produce only internal citrullines. Therefore, such peptides would be neoantigens that could induce a T-cell response, but with the antibodies reacting with internal (non-citrullinated) amino acid sequences. Moreover, \textit{P. gingivalis} has been shown preferentially to bind fibrinogen and other immobilised extracellular matrix proteins in the presence of their soluble forms,\textsuperscript{35} which may be a colonisation or nutritional mechanism in the oral cavity and may explain why \textit{P. gingivalis} specifically targets fibrinogen.

Similarly, there was an increased frequency of antibodies to CParg. The mechanisms underlying these phenomena are uncertain, particularly in the case of CParg, because the sequence of the peptides in CCP and CParg has not been published. However, with both antigens, the reaction in the periodontitis sera was preferentially with uncitrullinated forms of the antigen.

There were also raised levels, but not a significantly raised frequency, of antibodies to both citrullinated and uncitrullinated vimentin peptides in our periodontitis population, although the statistical significance of antibodies to citrullinated vimentin was lost in multivariate analysis. Interestingly, there was a highly significant interaction between periodontitis and smoking for anti-vimentin antibodies. Among non-smokers, patients with periodontitis had significantly higher titres of anti-vimentin antibodies, compared with non-periodontitis patients, independent of confounders.

Our finding that the autoantibody response to periodontitis is restricted to non-smokers is intriguing. Smoking is a strong dose-dependent risk factor both for chronic periodontitis\textsuperscript{36} and tooth loss,\textsuperscript{37} as well as an established dose-dependent environmental risk factor for antibody positive RA and RA disease severity,\textsuperscript{38-44} which may be partly mediated through its effect on citrullination of peptides\textsuperscript{45} in the lungs of smokers. Major genetic susceptibility factors for RA (ie, HLA-DRB1 shared epitope and PTPN22) and smoking have recently been shown to be associated with anti-CEP-1 and anti-cit-vim-positive RA.\textsuperscript{26,47} In the present study, there was effect modification by smoking. Among non-smokers, patients with periodontitis had higher anti-CEP-1 titres than non-periodontitis patients, and all anti-CEP-1-positive individuals were non-smokers, suggesting that periodontal disease (periodontitis) per se, rather than smoking, is involved in the generation of reactivity against CEP-1 in patients with periodontitis. The anti-cit-vim and anti-cit-fib titres were also higher among non-smokers with periodontitis, although the differences did not reach statistical significance after adjusting for confounders. Our results may suggest that smoking-induced citrullination and the citrullination induced by PPAD (or neutrophil activity during periodontitis) are entirely different mechanisms. Furthermore, the interaction may be due to differences in periodontal pathophysiology between smokers and non-smokers. For example, it is well established that smoking suppresses gingival inflammation,\textsuperscript{48,49} and recent studies suggest that the periodontal microbiome in periodontitis is different in smokers compared to non-smokers.\textsuperscript{50} Moreover, cigarette smoke has been shown to antagonise antibody-mediated HOCl release in neutrophils,\textsuperscript{51} which may have downstream effects on neutrophil extracellular trap release.\textsuperscript{52} The mechanisms whereby periodontitis may ultimately lead to an ACPA response and RA are unknown. Our findings suggest that uncitrullinated peptides may break tolerance in periodontitis, independent of major confounders including age, sex and smoking.

Disease duration could be a key factor in antibody formation and antibody profile. The natural course of periodontitis is known to be that of a slowly progressing disease, which is typically asymptomatic. Given that the periodontitis group consisted mainly of patients with relatively advanced periodontal breakdown, it is likely that they had had chronic periodontitis for many years. Whether these antibodies occur early in the disease course of periodontitis is yet to be determined.

In RA, ACPA precede the appearance of clinically identifiable arthritis by several years,\textsuperscript{18-22} and epitope spreading occurs before disease onset, but does not expand after disease onset.\textsuperscript{18-22} This might explain the low frequency of antibody reactivity to the arginine-containing control peptides for \textit{a-enolase}, vimentin and fibrinogen in established RA, as reported in a study of samples from nearly 2000 RA cases.\textsuperscript{47} Therefore, if our theory is correct it would be predicted that antibodies to the non-citrullinated versions of RA autoantigens may well occur before the evolution of ACPA. Certainly, it would be well worth studying pre-disease serum samples not only for fine specificity antibodies to established citrullinated antigens but also for antibodies to their uncitrullinated (arginine) control peptides.

In summary, distinct antibody reactivity against both citrullinated and uncitrullinated peptides was observed between individuals with and without periodontitis, independent of major confounders, suggesting that uncitrullinated peptides break tolerance in periodontitis, with epitope spreading to citrullinated epitopes in the small proportion of patients that may evolve into RA.

Contributors PdP was involved in study conception and design, data analysis, interpretation of results and drafting the article. TD made contributions to data acquisition and analysis. MC and MM made contributed to data acquisition. PJV, PIC, CDB, IC and TD made contributions to study implementation, and were involved in revising the manuscript critically for important intellectual content. All authors gave final approval of the final version.

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Competing interests None.

Ethics approval Studies were approved by the NHS Research Ethics Service (REC ref 05/Q2707/252 and South Birmingham LREC/0405).

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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Westhoff G, Rau R, Zink A. Rheumatoid arthritis patients who smoke have a higher need for DMARDS and feel worse, but they do not have more joint damage than non-smokers of the same serological group. Rheumatology (Oxford) 2008;47:849–54.


Clinical and epidemiological research
Paper 5
Smoke exposure as a determinant of autoantibody titre in α₁-antitrypsin deficiency and COPD

A.M. Wood*, P. de Pablo*, C.D. Buckley*, A. Ahmad* and R.A. Stockley#

ABSTRACT: Liberation of elastin peptides from damaged lung may be a mechanism of autoimmune lung disease. Citrullination, and anti-citrullinated protein antibody formation occurs in smokers, but the role of smoking in autoantibody generation relevant to pulmonary disease is unclear.

Anti-elastin, anti-cyclic citrullinated peptide (anti-CCP) and anti-mutated citrullinated vimentin (anti-MCV) antibodies were measured in 257 subjects with α₁-antitrypsin deficiency (AATD), 113 subjects with usual chronic obstructive pulmonary disease (COPD) and 22 healthy nonsmokers. Levels were compared between groups, against phenotypic features and against smoke exposure.

Anti-elastin antibodies were higher in controls relative to AATD (p < 0.008) and usual COPD (p < 0.00001), and in AATD relative to usual COPD (p < 0.00001). Anti-elastin levels showed a threshold at 10 pack-yrs, being higher in those who had smoked less (p = 0.004). No relationships between antibody levels and clinical phenotype were seen after adjustment for smoke exposure. Anti-CCP antibodies were higher in usual COPD than AATD (p = 0.002) but the relationship to smoke exposure was less clear.

Smoke exposure is the main determinant of anti-elastin antibody levels, which fall after 10 pack-yrs. Local antibody complexes may be a better measure of elastin directed autoimmunity than circulating levels.

KEYWORDS: α₁-Antitrypsin deficiency, autoimmune disease, chronic obstructive pulmonary disease, smoking and health

The protease–antiprotease hypothesis of the pathogenesis of chronic obstructive pulmonary disease (COPD) concerns imbalances between proteases that digest elastin and other components of the extracellular matrix in the lung parenchyma, and antiproteases that protect [1–2]. The origin of this theory comes from the observation that patients with α₁-antitrypsin deficiency (AATD) develop early onset emphysema [3]. α₁-Antitrypsin (AAT) is an antiprotease, which acts predominantly to block the action of neutrophil elastase, a protease released by neutrophils. Neutrophil elastase is a serine protease, the first of three classes of protease important in COPD. The remaining two classes are the cysteine proteases, such as cathepsin-B, and the matrix metalloproteases (MMPs) [4]. In general, the serine and cysteine proteases are capable of degrading elastin and some forms of collagen [4], whilst MMPs have more of an effect on collagen, gelatin and laminin [2], all of which are components of the extracellular matrix of the lung.

Breakdown of the extracellular matrix, particularly elastin, in the lung is a key feature of both COPD and AATD, as shown by the presence of high levels of elastin breakdown products. A desmosine cross-link is unique to elastin and may be used as a marker of its degradation [5]. Desmosine and elastin peptides are elevated in the plasma, urine and sputum of COPD patients [6], whilst urinary levels positively correlate with the annual rate of decline in forced expiratory volume in 1 s (FEV₁) in smokers [7]. In general, elastin breakdown is greater in subjects with AATD at a given level of smoking because of a relative excess of neutrophil elastase activity in the lung [8], confirmed by elevated desmosine levels compared with usual COPD subjects [6]. Elastin breakdown has been proposed as a mechanism for generation of autoantibodies directed against elastin, which could in theory perpetuate and/or aggravate lung destruction [9]. In both AATD and COPD, the main stimulus to elastin breakdown is cigarette smoking, but studies of anti-elastin antibody prevalence in these conditions to date have been too small to assess smoke effects on antibody levels adequately [9–11]. Although the original report of circulating anti-elastin antibodies in COPD was...
Paper 6
Long-Term Impact of Early Treatment on Radiographic Progression in Rheumatoid Arthritis: A Meta-Analysis

AXEL FINCKH,1 MATTHEW H. LIANG,1 CARMEN MUGICA VAN HERCKENRODE,2 AND PAOLA DE PABLO1

Objective. Although early initiation of disease-modifying antirheumatic drugs (DMARDs) is effective in controlling short-term joint damage in individuals with rheumatoid arthritis (RA), the long-term benefit in disease progression is still controversial. We examined the long-term benefit of early DMARD initiation on radiographic progression in early RA.

Methods. We identified published and unpublished clinical trials and observational studies from 1966 to September 2004 examining the association between delay to treatment initiation and progressive radiographic joint damage. We included studies of persons with RA disease duration <2 years and DMARD therapy of similar efficacy during followup. The differences in annual rates of radiographic progression between early and delayed therapy were pooled as standardized mean differences (SMDs).

Results. A total of 12 studies met the inclusion criteria. The pooled estimate of effects from these studies demonstrated a significant reduction of radiographic progression in patients treated early (−0.19 SMD, 95% confidence interval [95% CI] −0.34, −0.04), which corresponded to a −33% reduction (95% CI −50, −16) in long-term progression rates compared with patients treated later. Patients with more aggressive disease seemed to benefit most from early DMARD initiation (P = 0.04).

Conclusion. These results support the existence of a critical period to initiate antirheumatic therapy, a therapeutic window of opportunity early in the course of RA associated with sustained benefit in radiographic progression for up to 5 years. Prompt initiation of antirheumatic therapy in persons with RA may alter the long-term course of the disease.

KEY WORDS. Rheumatoid arthritis; Antirheumatic agents; Disease progression; Structural joint damage.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes progressive joint destruction and disability and is currently without cure. It is the most common systemic rheumatic disease, affecting ~1% of the population (1). Radiographic joint damage correlates strongly with long-term functional decline in patients with RA, and therefore preventing progressive joint damage has become a key treatment objective (2,3). Until recently, the recommended therapeutic strategy was to start with nonsteroidal antiinflammatory drugs (NSAIDs) or low-dose glucocorticoids, and progressively introduce more potent antirheumatic therapies only if the NSAIDs and glucocorticoids were insufficient to control the disease (pyramid approach). In the last 15 years, treatment goals have evolved from a concept of symptom control to a concept of disease control (4). This has resulted in a more aggressive therapeutic approach with earlier introduction of disease-modifying antirheumatic drugs (DMARDs) (5), which have been proven to reduce structural joint damage.

The rationale for a prompt initiation of DMARDs in patients with RA is based on the idea that there is a critical period, a therapeutic window of opportunity, during early stages of the disease when treatment is more effective than later in the course of the disease. This concept covers a short-term effect with better disease activity responses and...
Paper 7
Utility of ultrasound joint counts in the prediction of rheumatoid arthritis in patients with very early synovitis

Andrew Filer,1,2 Paola de Pablo,1,2 Gina Allen,3 Peter Nightingale,4 Alison Jordan,5 Paresh Jobanputra,5 Simon Bowman,5 Christopher D Buckley,1,2 Karim Raza1,2

ABSTRACT

Objectives Early therapy improves outcomes in rheumatoid arthritis (RA). It is therefore important to improve predictive algorithms for RA in early disease. This study evaluated musculoskeletal ultrasound, a sensitive tool for the detection of synovitis and erosions, as a predictor of outcome in very early synovitis.

Methods 58 patients with clinically apparent synovitis of at least one joint and symptom duration of ≤3 months underwent clinical, laboratory, radiographic and 38 joint ultrasound assessments and were followed prospectively for 18 months, determining outcome by 1987 American College of Rheumatology (ACR) and 2010 ACR/European League Against Rheumatism criteria. Sensitivity and specificity for 1987 RA criteria were determined for ultrasound variables and logistic regression models were then fitted to evaluate predictive ability over and above the Leiden rule.

Results 16 patients resolved, 13 developed non-RA persistent disease and 29 developed RA by 1987 criteria. Ultrasound demonstrated subclinical wrist, elbow, knee, ankle and metatarsophalangeal joint involvement in patients developing RA. Large joint and proximal interphalangeal joint ultrasound variables had poor predictive ability, whereas ultrasound erosions lacked specificity. Regression analysis demonstrated that greyscale wrist and metacarpophalangeal joint involvement, and power Doppler involvement of metatarsophalangeal joints provided independently predictive data. Global ultrasound scores were inferior to minimal power Doppler counts, which significantly improved area under the curve values from 0.905 to 0.962 combined with the Leiden rule.

Conclusion In a longitudinal study, extended ultrasound joint evaluation significantly increased detection of joint involvement in all regions and outcome groups. Greyscale and power Doppler scanning of metacarpophalangeal joints, wrists and metatarsophalangeal joints provides the optimum minimal ultrasound data to improve on clinical predictive models for RA.

PATIENTS AND METHODS

Patients Fifty-eight patients with clinically apparent synovitis of at least one joint and inflammatory joint symptoms (inflammatory joint pain, and/or swelling and/or morning stiffness) of 3 months or less duration underwent baseline assessment and 18-month follow-up to determine diagnosis as previously described. Ethical permission was obtained and all patients gave written informed consent. Patients were classified as having RA, reactive arthritis, psoriatic arthritis or miscellaneous conditions according to established criteria. In order to compare the distribution of joint involvement with established RA, 22 patients with newly presenting, treatment-naive RA of over 3 months’ duration fulfilling 1987 ACR criteria were also recruited.
Clinical, laboratory and radiographic assessment

Patients underwent baseline 66 swollen and 68 tender clinical counts. Age, sex, symptom duration, early morning stiffness duration, medication, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor (RF) and anticyclic citrullinated peptide antibody (ACPA) status were recorded. In all but six patients, none of whom fulfilled the 1987 or 2010 criteria for RA or subsequently developed erosions, baseline conventional radiography of hands and feet was recorded, and the presence of erosions assessed in a blinded fashion by a single trained observer (AF).

Ultrasound assessment

Within 24 h of clinical assessment, patients underwent blinded ultrasound assessment in a temperature controlled radiology suite. Patients were asked not to discuss their symptoms. A systematic multiplanar greyscale and power Doppler ultrasound examination of 92 sites in 38 joints (table 1) was performed based upon standard EULAR reference scans20 using a Siemens Acuson Antares scanner (Siemens, Bracknell, UK) and multifrequency (5–13 MHz) linear array transducers. For power Doppler examinations, the pulse repetition frequency was adjusted to provide maximal sensitivity at the lowest possible value for each joint, resulting in a pulse repetition frequency of between 610 and 780. Examinations took between 50 and 60 min depending on disease extent and patient mobility.

Ultrasound findings of synovitis, power Doppler positivity and erosion were defined according to consensus definitions.12 20–22 Greyscale synovitis in metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints was graded from 0 to 3 based upon the system of Szkudlarek and colleagues;12 23 reclassifying the equivocal ‘minimal’ thickening grade as normal: grade 0, normal; grade 1, synovial thickening bulging over the line linking the tops of the periaricular bones; grade 2, grade 1 plus extension to one bone diaphysis; grade 3, grade 1 plus extension to both bone diaphyses. Synovitis in other joints was graded 0–3 as: 0, normal; 1, mild; 2, moderate; and 3, severe, in which grade 1 demonstrates synovial thickening in excess of the mean plus 2 SD of normal range when available.22

Table 1 Synovial intra-articular recesses and periarticular sites evaluated by ultrasound

<table>
<thead>
<tr>
<th>Joint</th>
<th>Recess or site</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP (1–5), PIP (1–5), MTP (2–5)</td>
<td>Dorsal recess</td>
</tr>
<tr>
<td></td>
<td>Lateral recess (PIP, MCP 1, 2, 5, MTP 5)</td>
</tr>
<tr>
<td></td>
<td>Volar recess (PIP)</td>
</tr>
<tr>
<td>Wrist</td>
<td>Interarcap recesses</td>
</tr>
<tr>
<td></td>
<td>Radiocarpal recesses</td>
</tr>
<tr>
<td></td>
<td>Ulnararcap recesses</td>
</tr>
<tr>
<td></td>
<td>Volar carpel recesses</td>
</tr>
<tr>
<td>Elbow</td>
<td>Anterior recess</td>
</tr>
<tr>
<td></td>
<td>Humeroarial joint</td>
</tr>
<tr>
<td></td>
<td>Humeroquinal joint</td>
</tr>
<tr>
<td></td>
<td>Posterior recess</td>
</tr>
<tr>
<td>Shoulder</td>
<td>Subdeltoit bursa</td>
</tr>
<tr>
<td></td>
<td>Posterior glenohumeral recess</td>
</tr>
<tr>
<td>Knee</td>
<td>Suprapatellar recess</td>
</tr>
<tr>
<td></td>
<td>Medial parapatellar recess</td>
</tr>
<tr>
<td></td>
<td>Lateral parapatellar recess</td>
</tr>
<tr>
<td></td>
<td>Medial femorobical joint line</td>
</tr>
<tr>
<td></td>
<td>Lateral femorobical joint line</td>
</tr>
<tr>
<td>Ankle</td>
<td>Anterior tibialital recess</td>
</tr>
<tr>
<td></td>
<td>Medial tibialital recess</td>
</tr>
<tr>
<td></td>
<td>Lateral tibialital recess</td>
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</tbody>
</table>

MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; PIP, proximal interphalangeal joint.

Effusion in the absence of synovial thickening was not classified as synovitis. Synovial hyperaemia was measured by power Doppler in each recess and the maximal score graded according to Szkudlarek et al23: 0, absence; 1, isolated signals; 2, confluent signals in less than half of the synovial area; and 3, confluent signals in more than half of the synovial area. The presence of joint erosion was measured as a binary variable. Global ultrasound indices for greyscale synovitis and power Doppler were calculated by adding scores from all joints. Global ultrasound counts were calculated by adding scores after converting individual joint grades to binary variables.

Statistical analysis

Analysis of data including logistic regression was performed using Stata 10. Comparison of clinical and ultrasound counts within and between groups was analysed using McNemar’s test and Fisher’s exact test, respectively. Other baseline clinical and ultrasound variables were compared between groups using Mann–Whitney U or Kruskal–Wallis tests. Intraobserver reliability was evaluated by blindly rescoring representative images of 20 patients for synovitis and power Doppler at least 5 months after initial scans, and analysed using κ statistics (see supplementary table S1, available online only).

RESULTS

Patient characteristics

Patients developing RA by 1987 criteria (VERA) were significantly older than those in other groups as expected (table 2). Male patients were overrepresented in this group (55%) compared with the general RA population. No patients in the study had received disease-modifying antirheumatic drugs at baseline. Two patients (both of whom developed RA) had received a short course of prednisolone for 5 and 14 days before recruitment. Both fulfilled ACR criteria at the time of recruitment. Two patients in the group developing non-RA persistent disease (VENRA) were RF positive; one had reactive arthritis, the other had psoriatic arthritis. Of five VENRA patients remaining unclassified, one was treated with methotrexate and another with hydroxychloroquine within 1 month of presentation. The comparison group of 22 patients with established RA had a median symptom duration of 28 (IQR 17–65) weeks and median age 55 (IQR 45–63) years: 73% were women, and 64% were ACPA and/or RF positive.

Global clinical and ultrasound assessment of patients

A total of 4640 sites in 2204 joints was included in the analysis. Proportionately more joints were found to be involved by ultrasound greyscale assessment than clinical examination in VERA (see table 2). Ultrasound assessment led to the reclassification of many patients between monarthritis, oligoarthritis and polyarthritis groups. In particular nine (69%) VENRA patients were reclassified as polyarthritis, and eight (50%) resolving patients with a clinical monarthritis were reclassified as oligo or polyarthritis. The distribution of subclinical joint involvement found by ultrasound greyscale assessment in six patients with persistent disease and a clinical monarthritis at presentation (who without erosions could not be classified as having RA by the 2010 criteria) is shown in supplementary figure S1 (available online only).

Effect of ultrasound assessment on joint involvement by region

Clinical involvement was defined by at least one joint in a given region being clinically swollen and ultrasound involvement
by the presence of greyscale synovial hypertrophy of at least grade 1. The impact of increased sensitivity of ultrasound was most marked in the large joints, wrists and metatarsophalangeal joints (figure 1, supplementary table S1, available online only). Among VERA patients, clinically silent involvement of the wrists, elbows, knees, ankles and metatarsophalangeal joints was identified significantly more often by ultrasound. In VENRA patients, metacarpophalangeal joint (p<0.05), wrist (p<0.05), elbow (p<0.05) and metatarsophalangeal joint (p<0.01) involvement was detected more often by ultrasound (supplementary table S2, available online only). Compared with groups with persistent outcomes, ultrasound detected less additional involvement in the resolving group at the wrist (p<0.05) and metatarsophalangeal joints (p<0.01). To investigate the low levels of clinically apparent metatarsophalangeal joint and ankle synovitis in VERA patients, we assessed a comparison group of patients with newly presenting RA of more than 3 months’ duration. Clinical involvement of the proximal interphalangeal, ankle and metatarsophalangeal joints was more overt in these patients (figure 1C), with significantly greater involvement of the metatarsophalangeal joints (p<0.05).

Detection of erosive disease in early arthritis using ultrasound

Ultrasonographic erosions of the hands or feet were detected in 26 joints of 13 very early arthritis patients (table 2). Using conventional radiography, only one erosion was visible in the wrist.

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Table 2  Baseline patient demographics and global clinical and ultrasound data

<table>
<thead>
<tr>
<th>Final diagnostic group by 1987 criteria</th>
<th>VERA</th>
<th>VENRA</th>
<th>Resolving</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>29</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Final diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>29 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PsA</td>
<td>0</td>
<td>5 (38)</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>SLE</td>
<td>0</td>
<td>2 (15)</td>
<td>0</td>
</tr>
<tr>
<td>Parvovirus related</td>
<td>0</td>
<td>0</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>Pseudogout</td>
<td>0</td>
<td>0</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>Reactive</td>
<td>0</td>
<td>1 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Septic</td>
<td>0</td>
<td>0</td>
<td>1 (6.25)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0</td>
<td>5 (38)</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Age†**</td>
<td>63 (19–82)</td>
<td>45 (18–83)</td>
<td>40 (23–75)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>13 (45)</td>
<td>9 (69)</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Symptoms duration, weeks†</td>
<td>7 (2–12)</td>
<td>6 (3–12)</td>
<td>4 (1–9)</td>
</tr>
<tr>
<td>Morning stiffness, min†</td>
<td>120 (30–360)</td>
<td>60 (0–240)</td>
<td>52.5 (0–240)</td>
</tr>
<tr>
<td>NSAID use, n (%)</td>
<td>20 (69)</td>
<td>8 (62)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>RF positive, n (%)</td>
<td>15 (52)</td>
<td>2 (15)</td>
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</tr>
<tr>
<td>ACPA positive, n (%)</td>
<td>14 (48)</td>
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<td>0</td>
</tr>
<tr>
<td>ESR (mm/h)†</td>
<td>25 (0–104)</td>
<td>24 (4–87)</td>
<td>21.5 (0–102)</td>
</tr>
<tr>
<td>CRP (mg/l)†</td>
<td>15 (0–102)</td>
<td>16 (0–83)</td>
<td>16 (0–244)</td>
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<td>Swollen joint count of 66†***</td>
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<td>Tender joint count of 68†*</td>
<td>9 (0–41)</td>
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<td>DAS28 score†</td>
<td>4.49 (2.20–6.28)</td>
<td>3.90 (1.66–6.27)</td>
<td>3.57 (1.36–4.94)</td>
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</table>

Patients meeting ACR criteria at baseline

| 1987 ACR criteria, n (%) | 12 (41) | 0 | 0 |
| USGS 1987 ACR criteria, n (%) | 16 (55) | 3 (23) | 1 (6) |
| 2010 ACR/EULAR criteria, n (%) | 24 (83) | 2 (15) | 0 |
| USGS 2010 ACR/EULAR criteria, n (%) | 27 (93) | 5 (38) | 2 (13) |

Clinical pattern, n (%)

| Monarthritis | 1 (3) | 6 (46) | 8 (50) |
| Oligoarthritis (2–5 joints) | 10 (34) | 6 (46) | 7 (44) |
| Polyarthritis (>5 joints) | 18 (62) | 1 (8) | 1 (6) |

USGS pattern, n (%)†

| Monarthritis | 0 | 1 (8) | 0 |
| Oligoarthritis | 1 (3) | 2 (15) | 11 (69) |
| Polyarthritis | 28 (97) | 10 (77) | 5 (31) |

US variables

| GS index*** | 35 (5–78) | 14 (2–38) | 6 (2–19) |
| GS count of 38*** | 15 (5–33) | 9 (1–20) | 3 (2–12) |
| PD index*** | 25 (5–60) | 9 (2–26) | 5.5 (0–16) |
| PD count of 38*** | 12 (3–29) | 5 (1–13) | 3 (0–10) |

Presence of erosions

| Radiographic hand/foot erosion, n (%) | 1 (3.5) | 0 | 0 |
| US hand/foot erosion, n (%) | 11 (38) | 2 (15) | 0 |
| Any US erosion, n (%) | 11 (38) | 2 (15) | 1 (6.25) |

†Median (range).

‡Clinical examination variables have been extended by adding ultrasonographic criteria of joint involvement (USGS grade ≥1) and erosion.

ACPA, anticyclic citrullinated peptide antibody; ACR, American College of Rheumatology; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; GS, greyscale ultrasound; NSAID, non-steroidal anti-inflammatory drug; PD, power Doppler; PsA, psoriatic arthropathy; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus; US, ultrasound.

*p<0.05, **p<0.01, ***p<0.001 Kruskal–Wallis test.
Impact of ultrasound measured variables on 1987 and 2010 RA criteria fulfilment

At baseline, the 1987 ACR criteria identified 12 out of 29 RA patients, and no patients in VENRA and resolving groups. Adding ultrasound data identified 16 RA patients but misclassified a further four patients (table 2). The 2010 criteria identified 26 patients (nine with ultrasound erosions) at baseline including two VENRA patients (table 2, supplementary table S3, available online only). The difference between these values is almost entirely accounted for by the 6-week rule, without which 1987
criteria identified 23 VERA patients at baseline. Extending the 2010 criteria by adding ultrasound data identified a further eight patients, including three with erosions (supplementary table S2, available online only). All eight were classified as RA regardless of erosions by increasing the 2010 ‘joints’ score from either two or three to the maximum five. Ultimately, at 18 months the 2010 criteria failed to classify as RA four out of 29 VERA patients, including one patient with ultrasound defined erosions.

**Sensitivity and specificity of clinical and ultrasound variables for RA by 1987 criteria**

Clinical and ultrasound variables were assessed by calculating sensitivity, specificity and area under the receiver operating characteristic curve (AUC) for 1987 RA as an outcome (table 3), using threshold grades of 1 or more or 2 or more for ultrasound variables. Shoulder, elbow, knee and ankle ultrasound involvement was discarded from the analysis because of lack of specificity for RA, despite increased sensitivity (supplementary table S2, available online only). Ultrasound variables generally improved sensitivity with some loss of specificity. Modifying this by imposing a higher threshold grade or requiring symmetry resulted in improved AUC values for ultrasound variables in metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joint regions compared with clinical equivalents (table 3). In this cohort, adding ultrasound to clinical variables in the 1987 ACR criteria increased sensitivity at a cost of specificity resulting in a drop in AUC. However, power Doppler variables performed better than greyscale variables. Furthermore, greyscale and power Doppler assessments of metatarsophalangeal joints exhibited improved sensitivity compared with clinical variables, whereas power Doppler variables retained high specificity for RA. By combining variables with 100% specificity for RA (ACPA positivity, metatarsophalangeal joint power Doppler grade of 2 or more involvement and a metacarpophalangeal joint greyscale count of 8 or greater), 76% of VERA patients were identified, including eight of the 15 ACPA-negative individuals.

**Logistic regression analyses**

Significant variables on univariate analysis were entered as explanatory variables in logistic regression models with an outcome of RA by 1987 criteria as the dependent variable, and the Leiden score as the independent variable. An AUC was constructed to assess contribution to the prediction of RA above the Leiden rule for different models (table 4). The AUC for the Leiden rule as a continuous variable was 0.905 in this sample, similar to the value derived by van der Helm-van Mil et al. Global greyscale and power Doppler counts increased the AUC for predicting RA (table 4), indicating that ultrasound counts provide independently predictive data over and above the Leiden score.

An important aim of this study was to identify joint regions with the greatest potential for use in predictive models. We systematically examined individual ultrasound variables in combination with the Leiden rule by logistic regression (table 4). This analysis precludes examination of variables with 100% sensitivity or specificity, which were therefore omitted. No proximal interphalangeal joint ultrasound variables functioned as independent predictors. However, highly sensitive variables such as greyscale and power Doppler involvement of metacarpophalangeal joints and wrists contributed additional predictive information. Moreover, highly specific ultrasound variables such as high grade greyscale wrist symmetry, high ultrasound counts of metacarpophalangeal joints and power Doppler involvement and symmetry of metatarsophalangeal joints are most likely to suggest a diagnosis of RA if not clinically apparent, and may be combined to enhance prediction (table 3). Deriving a minimal 12-joint power Doppler ultrasound score in a similar manner to Naredo et al increased the AUC in this analysis. By removing the (less specific) knee joint from this score, a significantly increased AUC of 0.962 was obtained (p<0.05, figure 2).

**DISCUSSION**

We have demonstrated a diagnostic benefit of the increased sensitivity of ultrasound in an early synovitis population. Ultrasound assessment results in a considerable shift in disease category from monarthritis to oligoarthritis and/or polyarthritis, that is greater than that reported in patients with longer disease durations. This suggests that the increased sensitivity of ultrasound may have a greater impact in the very early window. Comparing very early with later onset RA supported this, as joints such as the metatarsophalangeals joints were more evident clinically later in the disease course. The 2010 criteria proved, as expected, to be more sensitive at baseline than the 1987 criteria. However, they failed to classify all patients ultimately classified as RA by the 1987 criteria. Adding ultrasound variables to the new 2010 criteria classified more patients as RA, including several later classified as RA by the 1987 criteria, one with ultrasound erosions. This suggests that the detection of subclinical disease by imaging will similarly prove useful in optimising sensitivity and specificity of the 2010 criteria.

Global ultrasound counts improve sensitivity with some loss of specificity. However, ultrasound of large joints and proximal interphalangeals joints is not helpful in predicting RA by the 1987 criteria. Global ultrasound joint counts therefore increased the discriminating ability of the Leiden rule, but require significant scanning time, and performed worse than minimal counts by including non-discriminating joints. Harrison and Symmons showed in the NOAR cohort that persistent synovitis was predicted by the presence of RF, a tender joint count of greater than six and ankle synovitis. However, ultrasound detected significantly more knee and ankle disease in all disease groups in our cohort, with no predictive benefit.

Two further findings from our data are important: first, that scanning the metatarsophalangeals joints, wrists and metatarsophalangeal joints is likely to be of useful predictive value. Subclinical ultrasound metatarsophalangeal joint involvement in very early arthritis demonstrated very good specificity for RA by the 1987 criteria. Long established data show that erosive metatarsophalangeal joint disease occurs despite the absence of symptoms or signs. Such subclinical disease may manifest as a positive metatarsophalangeal joint squeeze test, a suggested screening tool for possible early RA. Second, power Doppler measurements have a uniquely high specificity for RA compared with other groups, particularly at the metatarsophalangeals joints, with combined metacarpophalangeal joint, wrist and metatarsophalangeal joint assessments providing excellent AUC values. These data are compatible with those of Freeston et al who examined associations of inflammatory arthritis in a mixed population of patients with arthralgia and arthritis, finding that high grade power Doppler had good sensitivity and specificity for persistence. Therefore, in addition to correlations with erosive progression, power Doppler may also have useful predictive power for RA. Of particular interest is the increased AUC value of power Doppler indices and counts compared with greyscale equivalents, suggesting that power Doppler has superior specificity for RA. Reducing the complexity of power
Doppler indices to 12 joints in the manner of Naredo et al.\textsuperscript{24} had the effect of increasing the AUC, suggesting that joints effective for monitoring disease activity also have good specificity as predictors for RA. Removing the knee joint from this index further improved the AUC, and has the advantage of reducing scanning time. This finding requires validation in larger studies.

The increased sensitivity of ultrasound for erosions was greater than that in the ESPOIR cohort\textsuperscript{31} and of a similar order.

Table 3: Sensitivity and specificity of clinical and ultrasound variables for the prediction of RA

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<tr>
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<th>Sensitivity</th>
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<th>Positive LR</th>
<th>Negative LR</th>
<th>PPV</th>
<th>NPV</th>
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ACPA, anti-cyclic citrullinated peptide antibody; ACR, American College of Rheumatology; AUC, area under the receiver operating characteristic curve; GS, ultrasound greyscale; LR, likelihood ratio; metacarpophalangeal joint (MCP) GS ≥1 involvement, MCP joint involvement with an ultrasound greyscale grade of at least 1; MCP joint GS count ≥8, at least eight MCP joints with GS involvement of grade ≥1; NPV, negative predictive value; PD, ultrasound power Doppler; PD10 index, summed power Doppler grades of MCP 2–3 joints, wrists and metatarsophalangeal (MTP) 2–3 joints; PIP proximal interphalangeal joint; PPV, positive predictive value; RA, rheumatoid arthritis; US, ultrasound.
Extended report

Table 4  Impact of ultrasound on prediction of RA: multivariate analyses

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<tr>
<th>Variables</th>
<th>p Value</th>
<th>AUC</th>
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<td>Leiden score</td>
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<td>0.905</td>
</tr>
<tr>
<td>Global US variables</td>
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<tr>
<td>Leiden</td>
<td>0.005</td>
<td>0.939</td>
</tr>
<tr>
<td>GS index</td>
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<td>0.936</td>
</tr>
<tr>
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<td>0.033</td>
<td>0.924</td>
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<tr>
<td>PD index</td>
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<td>0.942</td>
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<tr>
<td>PD count</td>
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<td>0.942</td>
</tr>
<tr>
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<td>0.005</td>
<td>0.952</td>
</tr>
<tr>
<td>PD12 index (after Naredo et al)(^{14})</td>
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</tr>
<tr>
<td>Leiden</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>PD10 index</td>
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</tr>
<tr>
<td>US joint counts by region</td>
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<td></td>
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<tr>
<td>Leiden</td>
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<td>0.939</td>
</tr>
<tr>
<td>PD hand count</td>
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<td>0.939</td>
</tr>
<tr>
<td>Leiden</td>
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<td>0.936</td>
</tr>
<tr>
<td>PD MCP count</td>
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<td>GS hand count</td>
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</tr>
<tr>
<td>MCP GS ≥1 symmetry</td>
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</tr>
<tr>
<td>MCP PD ≥2 symmetry</td>
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<tr>
<td>Wrist joints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leiden</td>
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<td>0.936</td>
</tr>
<tr>
<td>Wrist GS ≥2 involvement</td>
<td>0.013</td>
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<tr>
<td>Leiden</td>
<td>&lt;0.001</td>
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<tr>
<td>Wrist GS ≥2 symmetry</td>
<td>0.015</td>
<td>0.939</td>
</tr>
<tr>
<td>MTP joints</td>
<td></td>
<td></td>
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<tr>
<td>Leiden</td>
<td>0.001</td>
<td>0.933</td>
</tr>
<tr>
<td>MTP PD ≥1 involvement</td>
<td>0.026</td>
<td>0.933</td>
</tr>
</tbody>
</table>

AUC, area under the receiver operating characteristic curve; GS, ultrasound greyscale; GS count, sum of greyscale data when converted to a binary variable; GS index, sum of greyscale grades for all scanned joints; metacarpophalangeal (MCP) joint GS ≥1 involvement; MCP involvement with an ultrasound GS grade of at least 1; PD, ultrasound power Doppler; PD10 index, summed power Doppler grades of MCP joint 2–3 joints, wrists and metatarsophalangeal (MTP) 2–3 joints; RA, rheumatoid arthritis; US, ultrasound.

of magnitude to that seen by Wakefield et al.\(^{14}\) Although not the strongest predictive variable in our analysis, ultrasound erosions had a high specificity for RA of 95%, greater than that of radiographic erosions in the Leiden undifferentiated cohort (77%).\(^{32}\) Sonographers should therefore remain assured that scanning for ultrasound erosions is of significant value in confirming clinically suspected disease. Although a recent study presented ultrasound examination of the fifth metatarsophalangeal joint as a useful test to confirm diagnosis in RA with a mean disease duration of 15 months,\(^{35}\) examining the fifth metatarsophalangeal joint for erosions in our very early cohort detected only one out of 29 RA patients This test is therefore not of use in the very early window of disease.

This study has some shortcomings that we have sought to minimise. The low proportion of female RA patients compared with a normal population may be a chance finding within a small group. Any subtle gender-related differences in RA severity\(^{34}\) are unlikely to impact on the results of this study. In addition, two patients with persistent unclassified disease that could potentially have developed into RA were treated with disease-modifying anti-rheumatic drugs. Our findings should be viewed with this in mind, as to omit treatment would have been unethical. We have harmonised any grading used with published schemes so as to maximise the applicability of our findings, and have taken precautions to eliminate sources of bias, for instance by using temperature controlled facilities to minimise power Doppler variability. The main limitation relates to the small size of this initial cohort. The sample size required to produce diagnostic algorithms using ultrasound measures of synovitis with unbiased statistical methods would be considerable, and data from the present study should inform the design of future studies. We have demonstrated that scanning not only the metacarpophalangeal joints, but also the wrist and metatarsophalangeal joints with greyscale and power Doppler, is likely to provide the optimum ultrasound data to improve on clinical predictive models for RA, and have demonstrated the unique predictive specificity of metatarsophalangeal joint sonography and power Doppler measurement for RA. These are vital first steps in the development of validated predictive algorithms that include ultrasound variables.

**Figure 2**  Area under the receiver operating characteristic (ROC) curve for rheumatoid arthritis (RA) as an outcome using a 10-joint power Doppler index (summed power Doppler grades of metacarpophalangeal joints 2–3, wrists and metatarsophalangeal 2–3 joints) combined with the Leiden score (grey), versus the Leiden score alone (black). The 10-joint power Doppler index was entered as an explanatory variable in logistic regression analysis with an outcome of rheumatoid arthritis RA as the dependent variable and the Leiden score as the independent variable. The area under the ROC curve was compared using a non-parametric algorithm developed by DeLong et al.\(^{25}\)

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**Competing interests** CDB and KR have received grants and honoraria from Wyeth, Cellzome, UCB and Pfizer. AF has received grant support from Cellzome and Pfizer. SB has received honoraria or grant support from Roche, Genentech, UCB,
REFERENCES


Paper 8
Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort

Mohammed Z Cader,1 Andrew Filer,1,2 Jonathan Hazlehurst,1 Paola de Pablo,1,2 Christopher D Buckley,1,2 Karim Raza1,2

ABSTRACT
Objective Early identification of patients with rheumatoid arthritis (RA) is essential to allow the prompt institution of therapy. The 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria, which replace the 1987 classification criteria, have been developed to facilitate such identification in patients with newly presenting inflammatory arthritis. This study therefore assesses the performance of these new criteria in patients with early synovitis.

Methods Data were analysed from patients with synovitis seen within 3 months of the onset of inflammatory arthritis. Patients were followed for 18 months to determine outcomes, and data on the cumulative fulfilment of 2010 and 1987 criteria and therapy were recorded.

Results 265 patients were included in the study. 60 had alternative diagnoses at baseline. Of the remaining 205 patients, 20% fulfilled both 1987 and 2010 criteria, 3% fulfilled only 1987 criteria and 22% fulfilled only 2010 criteria at baseline. The 2010 criteria, when applied at baseline, detected more patients who eventually required disease-modifying antirheumatic drugs (DMARD) (65 (62%) vs 40 (38%); p<0.001), especially methotrexate (50 (68%) vs 31 (42%); p<0.01), within the first 18 months. However, more patients whose disease eventually resolved without ever requiring DMARD were classified at baseline as RA according to the 2010 criteria than with the 1987 criteria (16 (8%) vs 5 (2%); p=0.01).

Conclusion The 2010 ACR/EULAR criteria allow more rapid identification of patients requiring methotrexate compared with the 1987 ACR criteria when applied at baseline. However, overdiagnosis is an important issue to consider if these criteria are to be used in very early disease.

Rheumatoid arthritis (RA) is a chronic destructive disease. However, increasing evidence suggests that early treatment can modulate its natural history, significantly slowing the rate of disease progression and increasing the likelihood of achieving remission.1–5 The prompt diagnosis and treatment of RA is therefore crucial.6 Traditionally, classification of RA has been based on fulfilment of the 1987 American College of Rheumatology (ACR) criteria.7 These criteria were developed in patients with established RA of several years’ duration and it has been shown that they have poor sensitivity for the diagnosis of RA in patients with early synovitis. The 2010 ACR/European League Against Rheumatism (EULAR) criteria were therefore developed with the purpose of facilitating the early recognition of RA.8

An important aim of the new classification criteria was to identify individuals at high risk of persistent and destructive disease, who might benefit from disease-modifying therapy. Consequently, an important phase in the development of the criteria was the identification of factors, and their relative weights, which were associated with a clinical decision to start methotrexate within the first 12 months. This was carried out through analysis of data from 3115 patients, with no evidence of alternative diagnoses, from nine early arthritis cohorts.9 This was followed by the evaluation of case scenarios to determine the relative contribution of clinical and laboratory factors deemed to be important in influencing the probability of developing RA.10 Finally, the findings of these first two phases were integrated and the optimal cut-off for definite RA was established.11 This development process was therefore reliant upon the ability of experts to identify high-risk patients correctly and start treatment with methotrexate. Nevertheless the approach avoids the inherent circularity of developing new criteria from existing criteria.

The purpose of this study was to compare the performance of the 1987 and 2010 criteria in a very early synovitis cohort, comprising patients who presented within 3 months of the onset of inflammatory arthritis symptoms and who were systematically followed up to determine outcomes. This is an ideal population to study in this context because it includes patients who develop persistent RA, but were seen within a very short time frame after disease onset—the very situation for which the new criteria have been constructed. Importantly, our cohort was not used to develop the 2010 criteria, so is free from the inherent bias this would generate.

Validating criteria for RA is problematical given the absence of a gold standard pathology-based diagnostic test against which clinical criteria can be compared. For this reason, we initially sought to compare the 2010 criteria against the 1987 criteria. This comparison is crucial for several reasons. First, there is an extensive epidemiological and clinical trials literature that has been developed utilising the 1987 classification system. Ascertaining the degree of overlap between the criteria will help establish the extent to which previous research can be generalised to patients classified under the new system. Second, for the 2010 criteria to be useful, they must be capable of identifying RA more rapidly than the 1987 criteria. It is important to note that a positive
classification using the new criteria does not necessarily imply a clinical diagnosis of RA. However, in developing the 2010 criteria, the identification of patients at risk of persistent disease requiring disease-modifying antirheumatic drug (DMARD) therapy, in particular methotrexate therapy, was a key goal. We thus analysed whether the 2010 classification criteria were better able to identify patients who eventually required DMARD treatment.

PATIENTS AND METHODS

Patients were recruited from the rapid access early inflammatory arthritis clinic at Sandwell and West Birmingham Hospitals NHS Trust. Patients referred to the clinic by their general practitioners were seen within 2 weeks. Participants were included in the current study if: (1) they had clinically apparent synovial swelling at one or more joints at their initial assessment; (2) they were seen within 3 months of the onset of any symptom attributed by the assessing rheumatologist to inflammatory joint disease (pain, stiffness and/or swelling); and (3) they had been followed up for at least 18 months.

Data were collected on patient demographic variables, fulfilment of the 1987 ACR criteria (using the list format) and symptom duration. Tender (n=68) and swollen (n=66) joint counts were performed. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) antibody type 2 status were measured. These were used to calculate scores under the new 2010 EULAR/ACR criteria, on the basis of joint involvement, serology, acute phase reactants and symptom duration. Radiographs were performed of the hands and feet. Systematic clinical follow-up, including metrology assessment, was carried out at 1, 2, 3, 6, 12 and 18 months and CRP and ESR were measured at these time points. Data on therapy used during follow-up were also collected. Autoantibody status was measured at baseline only. Patients were classified as having self-limiting disease if at final follow-up (at 18 months) they had no clinical evidence of synovial swelling, were not taking DMARD and had not received glucocorticoid treatment in any form in the previous 3 months.

Several important points should be highlighted regarding our application of the 1987 and 2010 criteria. First, the 2010 criteria stipulate that patients with a likely alternative diagnosis such as systemic lupus erythematosus (SLE) are excluded from analysis. Moreover, this process is under continuous review such that during follow-up an increasing number of patients are excluded. Indeed, a patient initially meeting the 2010 classification criteria for RA at baseline will have their RA status reversed if it later becomes apparent that their symptoms are due to another condition. Such an exclusion process is not explicitly mentioned in the 1987 criteria, but is widely adopted in practice. We have therefore applied the same approach for both the 2010 and 1987 criteria. The second issue relates to the cumulative fulfilment of criteria. This issue is not explicitly addressed within either the 1987 or 2010 criteria, although cumulative fulfilment for the 1987 criteria is widely adopted in practice and we have allowed for cumulative fulfilment in both criteria sets. In the context of the 2010 criteria, the maximum score within each of the four domains is carried forwards during follow-up.

Data analysis was performed using the statistical package for social sciences, version 17.0. Patient groups classified using each system were compared in terms of demographic variables, serology, joint involvement, treatment and outcome after 18 months of follow-up. Differences in means were assessed using a two-tailed, unequal Student’s t test. Proportions were compared using a χ² test. The time to reach a diagnosis of RA (of patients diagnosed by both criteria) was compared using a Wilcoxon signed rank test. The latter data were presented graphically as a cumulative percentage of patients reaching an RA diagnosis in survival plot format. p Values less than 0.05 were considered significant. Patients gave their informed consent before inclusion in the programme. The study received ethical approval from the local research ethics committee.

RESULTS

Cohort characteristics

Two hundred and sixty-five patients were included in the study. One hundred and thirty-seven (52%) were women and the median age was 49 years (IQR 35–64 years). Patients were seen after a median symptom duration of 42 days (IQR 25–61); all were seen within 3 months. Patients had a median tender 28-joint count of 2 (IQR 1–6), tender 66-joint count of 3 (IQR 1–7), swollen 28-joint count of 2 (IQR 1–5) and swollen 66-joint count of 3 (IQR 1–5). Median ESR was 27 mm/h (IQR 11–53 mm/h) and median CRP was 21 mg/l (IQR 6–51 mg/l).

Classification of patients using 1987 ACR and 2010 ACR/EULAR criteria

As shown in figure 1, 60 patients had alternative diagnoses at baseline (reactive arthritis n=22, psoriatic arthritis n=12, gout n=10, pseudogout n=5, inflammatory bowel disease-related arthritis n=4, SLE n=2, sarcoidosis n=2, dermatomyositis n=1, Behcet’s disease n=1 and septic arthritis n=1). They were therefore excluded from RA classification criteria. Of the remaining 205 patients, 41 (20%) fulfilled both the 1987 and 2010 criteria at baseline assessment. There were also, however, patients only classified as RA using one or other criteria. At baseline, six of 205 (3%) patients were identified as RA only by the 1987 ACR criteria, but a greater number of patients (46 of 205 (22%)) were only classified using the 2010 criteria.

Fourteen patients in the cohort developed new symptoms, signs or other features during follow-up that led to an alternative diagnosis and therefore became excluded from the classification criteria during follow-up, although they were included in all baseline analyses. Of the remaining 191 patients, at some point during the 18-month follow-up, 76 (40%) patients fulfilled both the 1987 and 2010 criteria (not necessarily simultaneously). Sixteen (8%) patients were classified under only the 2010 criteria and 20 (11%) patients under only the 1987 criteria. As discussed above, when calculating the 2010 criteria score at follow-up, the maximum score from each of the four domains was carried forward. However, it should be noted that this ‘cumulative’ approach only resulted in one additional RA classification compared with a ‘non-cumulative’ analysis.

A comparison of group characteristics of patients classified using 1987 ACR and 2010 ACR/EULAR criteria

A direct comparison was made between patients classified as having RA using the 1987 and 2010 criteria (table 1). Among the 205 eligible patients in the cohort, the 2010 criteria identify far more patients at baseline compared with the 1987 criteria (87 (42%) vs 47 (23%), respectively; p<0.0001). Among patients requiring DMARD (n=105), these criteria also detect more patients at baseline for whom DMARD were prescribed by 18 months (65 (62%) vs 40 (38%), respectively; p<0.001), especially methotrexate (50 (68%) vs 31 (42%) out of 73 patients requiring methotrexate; p<0.01). Data regarding the sensitivity and specificity,
positive and negative predictive values and positive and negative likelihood ratios of the two sets of criteria when applied at baseline for subsequent DMARD use and methotrexate use are shown (table 2). Receiver operating characteristic curves for the identification of patients requiring DMARD and methotrexate based on the two sets of criteria when applied at baseline are also shown (see supplementary figure 1, available online only). The percentage of patients eventually requiring DMARD among

Figure 1 Flowchart of patients in the early arthritis cohort to compare classification of rheumatoid arthritis (RA) using the 1987 and 2010 criteria.

Table 1 Comparison of patients classified as having RA at baseline and after 18-month follow-up according to the 1987 and 2010 criteria

<table>
<thead>
<tr>
<th>At baseline</th>
<th>After 18-month follow-up</th>
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</thead>
<tbody>
<tr>
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<td>1987 Criteria (n=47)</td>
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<tr>
<td>Age, years; median (IQR)</td>
<td>60 (50–69)</td>
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<tr>
<td>Female; n (%)</td>
<td>24 (51)</td>
</tr>
<tr>
<td>RF positive; n (%)</td>
<td>26 (55)</td>
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<tr>
<td>Anti-CCP positive; n (%)</td>
<td>28 (60)</td>
</tr>
<tr>
<td>Eventual outcome (after follow-up); n (%)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Definitive non-RA diagnosis</td>
<td>5 (11)</td>
</tr>
<tr>
<td>DMARD use within first 18 months; n (%)</td>
<td>31 (66)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>9 (19)</td>
</tr>
</tbody>
</table>

Anti-CCP, anticyclic citrullinated peptide antibody; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.
those identified as RA at baseline is similar for the 2010 criteria and the 1987 criteria (65 (74%) vs 40 (85%), respectively; p=0.16). Importantly, more patients whose disease eventually resolved spontaneously were classified with RA according to the 2010 criteria than with the 1987 criteria (16 of 205 patients (8%) vs five of 205 patients (2%); p=0.01). This includes over a quarter of the patients classified as RA solely by the 2010 criteria at baseline (table 3). The proportion of patients fulfilling each set of criteria that were autoantibody positive was comparable.

After 18-month follow-up, the numbers of patients classified using the respective criteria were similar (96 patients with the 1987 criteria vs 92 patients with the 2010 criteria). These patients were also similar in age and gender. By this stage, fewer patients requiring DMARD were identified according to the 2010 criteria compared with the 1987 criteria, although the differences were not statistically significant. Data regarding the sensitivity and specificity, positive and negative predictive values and positive and negative likelihood ratios of the two sets of criteria, when applied during follow-up, for DMARD and methotrexate use are shown (table 2). There were seven more patients classified as RA based on the 2010 criteria who had a self-limiting illness (21% vs 13%). After follow-up, there were no patients with a definitive diagnosis other than RA because these patients had been excluded. The diagnoses of those patients with RA according to the 1987 and 2010 criteria at baseline that were no longer considered to have RA at follow-up were as follows: 2010 criteria: SLE (n=2), psoriatic arthritis (n=2), inflammatory bowel disease-related arthritis (n=1), Sjogren’s syndrome (n=1), gout (n=1); 1987 criteria: SLE (n=1), Sjogren’s syndrome (n=1), psoriatic arthritis (n=1).

Next, patients were divided into groups depending upon whether they fulfilled both, one or neither of the classification criteria at baseline (table 3). The ‘2010 only’ group contains an even mixture of seropositive and seronegative patients. Half of these patients were started on methotrexate in the first 18 months. However, 12 (26%) had a self-limiting illness. By contrast, it is interesting to note that only four (10%) patients fulfilling both criteria at baseline had a self-limiting illness. Furthermore, 27 (66%) of the patients fulfilling both criteria at baseline required methotrexate.

### Table 2: Sensitivity and specificity, positive and negative predictive values and positive and negative likelihood ratios of the 1987 ACR and 2010 ACR/EULAR classification criteria when applied at baseline and during follow-up for subsequent DMARD use and methotrexate use

<table>
<thead>
<tr>
<th>Characteristics of patient groups according to fulfilment of classification criteria at baseline</th>
<th>Analyses for criteria when applied at baseline</th>
<th>Analyses for criteria when applied during follow-up</th>
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</thead>
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<tr>
<td></td>
<td>DMARD use</td>
<td>Methotrexate use</td>
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<td>Sensitivity (%)</td>
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<td>Specificity (%)</td>
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<td>Positive predictive value (%)</td>
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<tr>
<td>Negative predictive value (%)</td>
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<tr>
<td>Positive likelihood ratio</td>
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<tr>
<td>Negative likelihood ratio</td>
<td>0.49</td>
<td>0.67</td>
</tr>
</tbody>
</table>

ACR, American College of Rheumatology; DMARD, disease-modifying antirheumatic drug; EULAR, European League Against Rheumatism.

### Table 3: Characteristics of patient groups according to fulfilment of RA classification criteria at baseline and after follow-up

<table>
<thead>
<tr>
<th>Characteristics of patient groups according to fulfilment of classification criteria at baseline</th>
<th>Characteristics of patient groups according to fulfilment of classification criteria after follow-up</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1987 Criteria only; n=6</td>
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<tr>
<td>Age, years; median (IQR)</td>
<td>65 (58–70)</td>
</tr>
<tr>
<td>Female; n (%)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>RF positive; n (%)</td>
<td>0 (0)</td>
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<tr>
<td>Anti-CCP positive; n (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Eventual outcome (after follow-up); n (%):</td>
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<tr>
<td>Definitive non-RA diagnosis</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Self-limiting illness</td>
<td>1 (17)</td>
</tr>
<tr>
<td>DMARD use within first 18 months; n (%)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (17)</td>
</tr>
</tbody>
</table>

Anti-CCP, anticyclic citrullinated peptide antibody; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.
respectively, p<0.01; figure 3B). It should be noted that some of these patients (although not excluded at baseline) did reach an alternative diagnosis, such as psoriatic arthritis, during follow-up. The results remain significant even if these patients are excluded.

Influence of symptom duration at presentation on fulfillment of classification criteria and DMARD/methotrexate necessity

Patients with a baseline classification of RA using the 2010 criteria had significantly longer median symptom duration at presentation than non-RA patients (45 days (IQR 35–67) vs 42 days (IQR 24–62); p=0.02). This is unsurprising given that the new criteria themselves include a score for duration of disease. However, even after stratifying for patients with a duration less than 6 weeks (ie, duration score of 0), patients with RA still had a significantly longer median symptom duration than those with non-RA disease (31 days (IQR 27–35) vs 23 days (IQR 14–30); p<0.01).

A comparison of time to classification and DMARD/methotrexate necessity

To assess whether the 2010 criteria offer a more rapid identification of RA in the early stages of disease, the time to meeting criteria for RA under both systems was assessed. This showed that patients are identified significantly earlier using the 2010 criteria (p<0.01; figure 3A).

Next we compared the proportions of patients requiring methotrexate identified by the different criteria at baseline. Of the total of 73 patients started on methotrexate, a significantly higher proportion of patients fulfilled the 2010 criteria than the 1987 criteria at baseline (50 (69%) and 31 (43%), respectively, p<0.01; figure 3B).
Many patients who eventually fulfill the classification criteria for RA according to the 1987 ACR criteria present with an undifferentiated arthritis. The need to identify those whose disease will progress to RA has prompted the development of predictive algorithms that have been validated. A recognition of the potential utility of criteria to identify early arthritis patients who will develop a persistent destructive disease unless treated appropriately has led to the development of the ACR/EULAR 2010 criteria.

Data from our cohort show that significantly more patients presenting with an inflammatory arthritis of less than 3 months’ duration are classified as RA at baseline according to the 2010 criteria, compared with the 1987 criteria. Some of this is explained by the 6-week symptom duration rule contained within the 1987 criteria, which prevents classification of patients as RA if seen within 6 weeks of the onset of their symptoms. However, 29 of the 46 (63%) patients only classified according to the 2010 criteria would not have been classified according to the 1987 criteria at baseline, even if the 6-week rule was disregarded. Do these additional patients, diagnosed only according to the 2010 criteria, really have RA? Importantly, 65% of these patients were positive for RF or anti-CCP antibody. Furthermore, 65% of these patients required DMARD therapy during follow-up. However, equally importantly, 12 (26%) of these patients had a disease that resolved without DMARD therapy over an 18-month follow-up period. Basing treatment decisions upon fulfillment of the 2010 criteria would thus lead to more rapid treatment in some patients for whom this is likely to be beneficial, at the cost of the overtreatment of significant numbers of patients. During the validation of the 2010 criteria three cohorts were studied from Leiden, Leeds and Toronto. Among the cohort participants who received methotrexate within the first year from symptom onset, the proportions with a score of 6/10 or greater ranged between 87% and 97%. This contrasts with only 50 of 73 (69%) in our cohort who received methotrexate within the first 18 months. Therefore, almost one third of patients who were deemed to require methotrexate during follow-up (and did not have an alternative rheumatological diagnosis at baseline) did not fulfill the 2010 criteria for RA at baseline.

The 2010 criteria were developed specifically to identify patients with RA at an early stage. It is, however, interesting to note that during follow-up there were 20 patients who fulfilled the 1987 criteria for RA who were not diagnosed according to the 2010 criteria. Despite the fact that none of these patients were positive for RF or anti-CCP antibody, 70% of them were commenced on a DMARD.

Our cohort is unusual in that participants have very short symptom durations (mean approximately 1.5 months). Given the well-recognized observation that symptom duration is an important predictor of disease persistence, this may partly explain why the 2010 criteria performed so well in the analysis and validation cohorts, and why such a large proportion (38%) of our cohort exhibited self-limiting disease without the requirement for DMARD. The mean symptom duration in the analysis and validation datasets used in development of the 2010 criteria (4.9 months) was longer than in our cohort, and only two of the datasets used in the development of these new criteria (from Austria and Norway) had mean symptom durations of under 3 months.

Our data suggest that the 2010 criteria will allow the more rapid identification of patients requiring methotrexate compared with the 1987 criteria if applied at baseline, but highlight that over and underdiagnosis may become important issues if these criteria are used to direct treatment within the phase when treatment makes the greatest difference—the first 3 months after symptom onset.

**Extended report**

**DISCUSSION**

Many patients who eventually fulfill the classification criteria for RA according to the 1987 ACR criteria present with an undifferentiated arthritis. The need to identify those whose disease will progress to RA has prompted the development of predictive algorithms that have been validated. A recognition of the potential utility of criteria to identify early arthritis patients who will develop a persistent destructive disease unless treated appropriately has led to the development of the ACR/EULAR 2010 criteria.

Data from our cohort show that significantly more patients presenting with an inflammatory arthritis of less than 3 months’
Ethics approval This study was conducted with the approval of the Birmingham East North and Solihull Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES
Paper 9
Performance of an Automated Computer-Based Scoring Method to Assess Joint Space Narrowing in Rheumatoid Arthritis

A Longitudinal Study

Axel Finckh,1 Paola de Pablo,2 Jeffrey N. Katz,2 Gesa Neumann,2 Ying Lu,3 Frederick Wolfe,4 Jeffrey Duryea2

Objective. To compare the diagnostic performance of a computer-based method for measuring joint space width with the Sharp joint space narrowing (JSN) scoring method in patients with rheumatoid arthritis (RA).

Methods. A random sample of patients with early RA, for whom sequential hand radiographs and Sharp scores were available, was selected from the National Data Bank for Rheumatic Diseases. Hand joint space width was measured using an automated, computer-based method in random order and with blinding for clinical information. We constructed a receiver operating characteristic curve and compared the diagnostic performance of the computer-based and Sharp methods based on the areas under the curve.

Results. One hundred twenty-nine patients with early RA who underwent serial radiography were included. Changes in the computer-based and Sharp methods were highly correlated ($r = 0.75, P < 0.001$). The computer-based method was significantly more discriminant than the Sharp JSN subscale. The area under the curve of the computer-based method was 0.96 (95% confidence interval [95% CI] 0.94, 0.99) compared with 0.93 (95% CI 0.89, 0.96) for the Sharp subscale ($P = 0.024$). At the most discriminant cutoff, specificity was 81.4% for the computer-based method compared with 81.4% for the Sharp subscale ($P = 0.11$); sensitivity was 87.6% for the computer-based method compared with 82.2% for the Sharp subscale ($P = 0.19$). The signal-to-noise ratio for the computer-based method was 83% compared with 70% for the Sharp subscale ($P = 0.013$).

Conclusion. The computer-based method for measuring joint space width is more discriminant than the semiquantitative Sharp JSN subscale.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that leads to progressive joint destruction, functional disability, and extraarticular complications. Structural joint damage correlates with long-term functional decline in RA patients (1). Thus, controlling progressive joint damage has become a key treatment objective (2). Conventional radiography permits measurement of structural joint damage, and films can be masked or randomized for standardized damage scoring. Radiographic measures of structural joint damage are currently considered the gold standard of treatment efficacy studies in RA (3), and are used extensively in clinical trials as the primary outcome measure. Fur-
Paper 10
Objective. Computer-based methods to measure radiographic joint space width (JSW) have the potential to improve the longitudinal assessment of rheumatoid arthritis (RA). The purpose of this report was to measure the long-term patient repositioning reproducibility of software-measured radiographic JSW.

Methods. Patients underwent baseline and followup hand radiography examinations with a followup time of ≤3 years. To eliminate any JSW change due to real disease progression, the evaluation was performed on “unaffected” joints, defined as having JSW and erosion Sharp scores of 0 at both baseline and followup. The root mean square SD (RMSSD) and coefficient of variation (CV) were used as the reproducibility metrics.

Results. The RMSSD was 0.14 mm (CV 10.5%) for all joints, 0.18 mm (CV 10.9%) for the metacarpophalangeal (MCP) joints, and 0.08 mm (CV 8.3%) for the proximal interphalangeal (PIP) joints. The distribution of JSW change was asymmetric, suggesting that narrowing due to RA progression occurred for several joints. A second analysis was performed, excluding joints where the loss of JSW was greater than 3 SDs. For this analysis, the RMSSD was 0.10 mm (CV 7.5%) for all joints, 0.12 mm (CV 7.3%) for the MCP joints, and 0.07 mm (CV 7.1%) for the PIP joints.

Conclusion. Repositioning reproducibility is very good but is likely to be a dominating factor compared to reader and software reproducibility. Additionally, further evidence is given that a software method is able to detect changes in some joints for which the Sharp score is insensitive.

INTRODUCTION

Radiography is used routinely to monitor progression in common and potentially disabling diseases such as rheumatoid arthritis (RA) and osteoarthritis (1,2). Radiographic change is considered the “gold standard” to assess disease progression in RA and is a common outcome measure for clinical trials (3).

There are two main structural changes from RA visible on conventional radiographs: 1) increase in erosion size and number and 2) loss of joint space width (JSW). Erosions, i.e., cavities created in the bone near the joint, are seen as radiolucent or dark regions or discontinuities in the bone margins. JSW is an indirect measure of cartilage loss, and can be appreciated on radiographs by a decrease in the distance between the projected margins of a joint.

Research requires reproducible and quantitative surrogate outcome measures; however, radiographic assessment using traditional scoring methods such as the Sharp (4) and Larson and Thoen (5) systems is subjective and based on a qualitative assessment of the joints. The available scoring methods do not attempt to provide a true measure of the size of the radiographic structures; rather, a score is given on an ordinal scale that is based on a comparison to representative examples.

Image analysis software can be used to provide quantification of these structural changes on a continuous scale and has been shown to be more responsive to change than semiquantitative scoring (6). Computerized methods also provide automated archiving of scores and integrate directly with digital imaging modalities. On the other hand, software is usually not 100% reliable and a quality assurance step is generally needed to ensure that the structures of interest are accurately identified. The need for a quality assurance step necessarily implies a degree of measurement error associated with the reader and correction software.

Several computer-based methods to measure radio-
Paper 11
Reproducibility of a Computer-Based Scoring System to Measure Joint Space Width on Digital Hand Radiographs of Subjects with Rheumatoid Arthritis

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Abstract

Objective. To evaluate the reproducibility of a computer-based method to measure the radiographic joint space width (JSW) in patients with rheumatoid arthritis.

Methods. We used hand radiographs from a random sample of patients with RA from the National Data Bank for Rheumatic Diseases. The semi-automated computer-based scoring system automatically delineated joint margins on MCP and PIP joints to measure the JSW. Four readers independently evaluated digitized hand radiographs from patients with RA on two separate occasions. Reader time was recorded. Inter-rater and intra-rater reproducibility was assessed with intraclass correlation coefficients (ICC), root-mean square standard deviation (RMSSD) and coefficients of variation (CV).

Results. We assessed the hand radiographs from a random sample of patients with RA using the software method. Average reader time was 36 seconds per joint and 4.4 minutes per hand. Mean JSW was 1.65 mm and 1.03 mm for MCP and PIP joints, respectively.

The inter-rater ICC values ranged between 0.96 and 0.98, and the RMSSD between 0.03 and 0.036. The CV was 2.2% and 2.9% for MCP and PIP joints, respectively.

Regarding intra-rater reliability, the ICC ranged between 0.95 and 0.97, and the RMSSD between 0.02 and 0.031 mm. The CV was 1.8% and 2.2% for MCP and PIP joints, respectively.

Conclusion. The proposed software method for JSW measurements has good reproducibility and a short reader time. This system has the potential for use in large clinical studies and should provide a quantitative, reproducible and more objective outcome measure of structural damage and response to therapy.

Key words: rheumatoid arthritis; radiograph; scoring; joint space width measurement; automated measurement; computer-based; reliability; reproducibility.
Paper 12
Antioxidants and Other Novel Cardiovascular Risk Factors in Subjects With Rheumatoid Arthritis in a Large Population Sample

PAOLA DE PABLO, THOMAS DIETRICH, AND ELIZABETH W. KARLSON

Objective. To compare antioxidants and other novel and traditional cardiovascular disease (CVD) risk factors in participants with rheumatoid arthritis (RA) and non-RA controls in a large population sample.

Methods. The Third National Health and Nutrition Examination Survey (NHANES-III) was a cross-sectional population survey in which subjects ages ≥60 underwent a musculoskeletal examination. RA subjects were defined as those who met ≥3 of 6 available 1987 American College of Rheumatology (ACR) criteria. Non-RA subjects were defined as those who met no ACR criteria. We performed univariate and multivariate analyses of the association between RA and each novel and traditional CVD risk factor in RA versus non-RA subjects.

Results. The sample included 5,302 subjects ages ≥60, with 131 (2.5%) RA and 4,444 (84%) non-RA participants. A total of 727 subjects were excluded. Plasma levels of antioxidants α-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene were significantly lower in RA subjects compared with non-RA subjects in multivariate analysis adjusting for potential confounders. Compared with non-RA participants, RA subjects were more likely to have increased C-reactive protein (CRP) levels in multivariate analysis adjusting for potential confounders. RA and non-RA participants had similar prevalence of traditional CVD risk factors and previous CVD.

Conclusion. In this large population study, RA subjects had similar prevalence of previous CVD and traditional CVD risk factors as controls. Among novel CVD risk factors, plasma carotenoid levels were significantly lower and CRP level was significantly higher in RA compared with non-RA subjects after adjustment for potential confounders. Further research should evaluate whether these differences account for the observed increased incidence of CVD in individuals with RA.

KEY WORDS. Rheumatoid arthritis; Antioxidants; Cardiovascular risk factors.

INTRODUCTION

Rheumatoid arthritis (RA), the most common systemic, chronic inflammatory, autoimmune disease, is associated with excess cardiovascular morbidity and mortality that is not entirely explained by traditional risk factors for cardiovascular disease (CVD) (1–4). Novel risk factors for CVD including inflammatory biomarkers, antioxidants, and/or vitamins may contribute to excess CVD in persons with RA.

RA and atherosclerosis share common inflammatory mechanisms (5,6). There is an association between inflammatory markers and subsequent CVD in healthy subjects (7–10). Therefore, chronic systemic inflammation may contribute to the higher incidence of CVD in persons with RA (11–14).

Moreover, there is a complex relationship between inflammation, antioxidant vitamin status, and the risk of CVD. Previous reports have demonstrated inverse associations between inflammation and antioxidant serum levels, in particular carotenoids (15–17). Furthermore, intakes of antioxidant vitamins and other micronutrients or their serum concentrations, including carotenoids (18–20), vitamin C (21–23), vitamin E (24,25), and folate and homocysteine (26–28), have been reported to be inversely associated with CVD incidence and mortality.
Is there a relationship between uric acid and inflammation in the U.S. population?

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Abstract

Objectives: Studies, albeit inconsistent, have suggested that serum uric acid (SUA) may have a direct role in atherogenesis and experimental data support a proinflammatory role of SUA. However, there is little data from clinical and epidemiologic studies on an association between SUA and inflammation. The objective was to evaluate the association between serum SUA and inflammation as measured with C-reactive protein (CRP).

Design and setting: The National Health and Nutrition Examination Surveys (NHANES), conducted between 1999 and 2004 in the USA, was a cross-sectional study of a nationally representative sample of non-institutionalized adults aged 20+ years.

Participants: The study sample included 10,376 participants with complete data. There were 4,942 men and 5,434 women. A priori, participants taking gout medications and those with hypouricemia were excluded from analyses.

Main outcome measure: CRP. We defined SUA into separate categories for men and women according to serum concentrations defined as normal low (reference), normal high, and high (hyperuricemia). We performed linear regression analyses of log transformed CRP on SUA, controlling for potential confounders, in separate models for men and women.

Results: Men had mean higher SUA and lower CRP concentrations compared with women (SUA 6.01±1.3 vs. 4.71±1.3 mg/dl and CRP 0.38±0.88 vs. 0.55 ±0.86 mg/dl, respectively). There was a positive linear dose-dependent association between SUA and CRP concentrations. The associations were strongest in participants with BMI<25 both in men (Δ 17%, 95% CI 11 to 25) and women (Δ 31%, 95% CI 21 to 42), and attenuated with increasing BMI among individuals with overweight or obesity (p for interaction: p<0.0001).

Conclusions. Even within the normal range, SUA concentrations are independently associated with CRP among men and women, in particular in normal weight and overweight individuals. If these results are confirmed in longitudinal studies, strategies to modify SUA should be tested to see whether they modify the long term risk of cardiovascular disease.
Paper 14
Association between Uric Acid and Inflammation in Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is associated with cardiovascular morbidity and mortality. C-reactive protein (CRP) is an independent predictor of future vascular events in the general population. Studies have suggested that serum uric acid (SUA) may have a direct role in atherogenesis and epidemiologic studies have observed an independent association between SUA and CRP. However, it is unclear whether this association holds in the context of a “high-grade” chronic inflammatory disease, such as RA. The objective was to evaluate the association between concentrations of SUA and CRP in a cohort of patients with RA.

Methods: We examined a cohort of 400 patients with RA recruited from routine outpatient clinics. We fitted fractional polynomial regression to evaluate the association between SUA and CRP, adjusting for potential confounders.

Results: After excluding participants with concomitant gout, the study sample included 381 participants with RA. Of those, 74% were female, 76% were seropositive, 70% were hypertensive and 10% had diabetes. Mean age was 61 years (SD±12) and mean disease duration was 12.5 years (SD±10.5). Men had higher SUA and CRP concentrations than women (6.0±1.3 vs. 4.9 ±1.5 mg/dl and 17.3±20.8 vs. 16.8±23.5 mg/dl, respectively). There was a non-linear association between SUA and CRP concentrations (overall p-value 0.01) independent of age, gender, renal function, and other potential confounders.

Conclusions: SUA concentrations are independently associated with inflammation in patients with RA. The physiologic basis of this association, as well as its significance in terms of the articular and vascular phenotype of RA require further exploration.
Paper 15
Association Between Bone Mineral Density and C-Reactive Protein in a Large Population-Based Sample

Paola de Pablo, Mark S. Cooper, and Christopher D. Buckley

Objective. Several studies suggest that bone mineral density (BMD) is reduced in chronic inflammatory diseases. Higher serum levels of C-reactive protein (CRP) have been associated with lower BMD in women and older adults. However, it is not clear whether this association holds in a representative sample of the general population. The purpose of this study was to examine the relationship between BMD and CRP level in a large representative US population-based sample from the National Health and Nutrition Examination Survey (NHANES).

Methods. We included participants age ≥20 years with BMD (total and subregions) measured by dual x-ray absorptiometry scans and complete information on covariates from NHANES. The association between CRP level and BMD was evaluated using multivariate linear regression models, adjusting for potential confounders and further adjusting for comorbid diseases, medications, and serum vitamin D levels.

Results. The study sample included 10,475 participants (53% Caucasian, 22% Mexican American, 18% African American, and 7% other races). Men had higher BMD and lower CRP concentrations than women. BMD (total body BMD as well as subtotal BMD and BMD of the extremities, ribs, and trunk subregions) was inversely associated with quintiles of CRP concentration both in men and in women in a dose-dependent manner (for total BMD, P for trend < 0.0001 for men, P for trend = 0.0005 for women). The associations were independent of medications, comorbidities, and other potential confounders. The results remained largely unchanged with further adjustment for serum vitamin D levels.

Conclusion. Among men and women in a large representative population-based sample, the CRP level was inversely and independently associated with total BMD in a dose-dependent manner.

Chronic inflammatory diseases are associated with systemic bone loss (1), osteoporosis, and increased risk of nontraumatic fracture. Such diseases include rheumatoid arthritis (RA) (2,3), ankylosing spondylitis (4), systemic lupus erythematosus (5), inflammatory bowel disease (6,7), and chronic obstructive pulmonary disease (COPD) (8). In this context, the pathogenic damage to bone is mediated by the interaction of inflammatory cells, cytokines, and bone cells. For example, in RA, generalized bone loss is related to increased osteoclast activity mediated by inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6, through the RANKL/RANK/osteoprotegerin system (9,10). Bone loss reversal has been observed with anti-TNF therapy, with some studies showing increments in spinal and femoral bone mineral density (BMD) (6,11–17).

While the association between chronic inflammatory diseases and bone loss, osteoporosis, and increased fracture risk is well established, data are scarce regarding whether low-grade inflammation has a similar effect or whether there is a dose-response relationship between inflammation and bone loss in the general population. Previous epidemiologic studies of the association between biomarkers of inflammation and BMD or bone loss have provided inconsistent or even contradictory results, with 2 studies showing an inverse association between C-reactive protein (CRP) level and BMD among Korean women and Southern Tasmanian older adults (18,19), and other studies showing no independent as-
Statements of authors’ contribution
Statement of authors’ contribution on the following publications


PdP conceived the study on the aspect of ACPA reactivity among individuals with lung disease, made contributions to the study design and was involved in revising the manuscript critically for important intellectual content. PdeP made substantial contributions to acquisition of data regarding ACPA reactivity.


PdP formulated the analysis plan and performed the statistical analysis. PdP produced the tables and figures. PdP was involved in revising the manuscript critically for important intellectual content. AF and KR made substantial contributions to acquisition of data and conceived the study. All authors read and approved the final manuscript.

PdP made contributions to the study conception, statistical analysis and was involved in revising the manuscripts critically for important intellectual content. MZC, JH, AF and KR made substantial contributions to acquisition of data. All authors read and approved the final manuscript.

Yours sincerely

[Signature]

Professor Christopher Buckley
Clinical Director Center for Translational Inflammation
Arthritis Research UK Professor of Rheumatology
To whom it may concern:

The following is a statement of Dr. Paola de Pablo’s contribution to the following publications.


Paola de Pablo carried out radiographic assessments of joint space width, made contributions to the study design, and was involved in revising the manuscripts critically for important intellectual content. She also made significant contributions to the development of the primary methodology used to perform the study that produced the publications.

Sincerely,

Jeffrey Duryea