Predictive Factors for the Development and Disease Course of Rheumatoid Arthritis

Annemarie Krabben

ISBN: 978-94-6169-559-8

The research presented in this thesis was performed at the department of Rheumatology at the Leiden University Medical Center, Leiden. The research was financially supported by the Dutch Arthritis Foundation (Reumafonds).

Cover: Studio57 Grafisch Ontwerp
Lay-out and printing: Optima Grafische Communicatie

The publication of this thesis was financially supported by the Dutch Arthritis Foundation (Reumafonds), Abbvie and Pfizer B.V.

Predictive Factors for the Development and Disease Course of Rheumatoid Arthritis

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof. Mr. C.J.J.M. Stolker,
volgens het besluit van het College voor Promoties
ter verdediging op 30 oktober 2014
klokke 13:45 uur

door

Annemarie Krabben

geboren te Rotterdam
in 1983

PROMOTIECOMMISSIE:

Promotor:	Prof. dr. T.W.J. Huizinga
Co-promotoren:	Dr. A.H.M. van der Helm-van Mil Dr. M. Reijnierse
Overige leden:	Prof. dr. R.E.M. Toes Prof. dr. W.F. Lems (VUMC, Amsterdam) Prof. dr. ir. B.P.F. Lelieveldt

TABLE OF CONTENTS

PART IV MRI scan protocol revisited

1

General introduction

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is an immune-mediated inflammatory systemic disease. RA affects 0.5-1% of the civilized population, three times more women than men and the prevalence rises with age.[1] RA has a clinical manifest of symmetric polyarthritis of especially the small hand and foot joints and can progress into a very immobilizing and disabling disease, without effective treatment and leads on socio-economic perspective to high costs and loss of work force.

RA is an auto-immune disease, with inflammation against own tissue in primarily the joints. Due to interaction between T cells, B cells, macrophages and fibroblast-like synoviocytes several pro-inflammatory cytokines are overproduced, like; tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6) and B cells start producing antibodies. These inflammatory cascades lead towards persistent synovial inflammation and destruction of cartilage and bone.[1] However, whether rheumatoid arthritis starts from inside the bone and spreads further into the tissue in the joint, or it starts with inflammation of the joint tissue which leads to bone involvement from outside, still remains unknown.[2]

RA is characterized by auto-antibodies like Rheumatoid Factor (RF) and anti-citrullinated peptide antigens (ACPA). ACPA and RF have a high specificity (80-90%) and a moderate sensitivity for the diagnosis of RA.[3] Approximately 55% of the RA patients are RF positive and 60% ACPA positive.[4-6] In addition, ACPA and RF positivity is also important for the disease course, because these are risk factors for a persistent and more destructive disease.[7] Furthermore, from previous studies we learned that these auto-antibodies are a median of 5 years present before the first symptoms of RA start and thus precede the clinical presentation phase of RA.[8] This might suggest that there is a pre-clinical stage present in RA.

From literature we learned that early recognition and treatment will result in a better outcome.[9,10] Some studies even suggest that treatment within this early 'window of opportunity' might alter the natural course of RA.[11]

UNCLASSIFIED ARTHRITIS

A patient with at least one swollen joint has arthritis. Together with other clinical symptoms, results from blood tests, imaging and other techniques the Rheumatologist will try to classify this patient. When all diagnoses, such as osteoarthritis, gout, reactive arthritis, spondyloarthropathy, systemic lupus erythematosis and of course rheumatoid arthritis are excluded, the patient can be classified as unclassified arthritis (UA).[12] Therefore UA is called a diagnosis per exclusion. 35% to 54% of the arthritis patients are classified as UA in the early arthritis cohorts.[5,13] However, the frequency is dependent on the duration of symp-

toms. Especially UA patients with a monoarthritis or oligoarthritis could not be classified as RA. Patients with unclassified arthritis can progress into rheumatoid arthritis (70% of the ACPA positive UA patients within 1 year) over time or can achieve remission or self-limiting disease.[14] The percentage of remission (40%-55%) in unclassified arthritis patients is larger than the percentage of remission in rheumatoid arthritis (10%-15%) and also the time to remission is shorter in unclassified arthritis patients.[14,15] This all suggests that the group of UA patients is a very heterogeneous group of patients and that remission is less common when the disease is more mature. However, UA can be pre-RA, but can also be a different disease and never progress to RA (Figure 1).

Figure 1. Disease phases of RA. Patients do not have to pass through all phases before RA eventually develops.

ARTHRALGIA

When taking a look even earlier in the course of the disease, even before the patient has arthritis, patients generally experience a period in which they have tender or painful joints, without the presence of a swollen joint. This is called arthralgia. Arthralgia can be accompanied by other symptoms. Some of these symptoms and characteristics about that patient with arthralgia can make the patient more suspect to finally progress into rheumatoid arthritis. The presence of ACPA and/or RF does increase the risk of arthritis development. A prediction model defined several additional risk factors for arthritis development in ACPA+ and/or RF arthralgia patients.[16] ACPA positive arthralgia patients have a 30% chance of RA development within 1 year.[17]

CLASSIFICATION CRITERIA AND PREDICTION MODELS FOR RA DEVELOPMENT

In 1987 a set of criteria has been developed to classify patients with arthritis as having rheumatoid arthritis.[18] These classification criteria were soon implanted as a diagnostic tool for Rheumatologists. However, the focus of diagnosing RA became more and more on early diagnosing and this criteria set is lacking in classifying early RA patients the 2010 classification criteria have been developed to classify more early RA patients.[19] Although,

officially these criteria are classification criteria to discriminate patients with and without RA in order to include in trials and to have a common definition in research communication and not to define patients who need early treatment, these criteria are more and more implanted in daily clinical practice (Figure 2).

These new criteria have resulted in classification of more early RA patients, but likewise a more heterogeneous group of RA patients with some over classification (patients with self-limiting disease).[4,6,20-22] These new criteria not only higher the prevalence of RA and

1987 criteria (4 out of 7)
1. Morning stiffness
2. Arthritis of 3 or more joint areas
3. Arthritis of hand joints
4. Symmetric arthritis
5. Rheumatoid nodules
6. Serum rheumatoid factor
7. Radiographic changes
*and at least 6 weeks symptom duration

2010 criteria (score ≥6/10)	
1. Joint involvement (0-5)	
1 large joint	0
2-10 large joints	1
1-3 small joints	2
4-10 small joints	3
>10 joints (at least 1 small)	5
2. Serology (0-3)	
ACPA neg and RF neg	0
ACPA low-pos and RF low-pos	2
ACPA high-pos and RF high-pos	3
3. Acute-phase reactants (0-1)	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
4. Duration of symptoms (0-1)	
<6 weeks	0
≥6 weeks	1
*patients with RA typical erosions should be classified as having RA	
*synovitis not better explained by another disease	

Figure 2. 1987 and 2010 classification criteria for RA, in patients with arthritis.

lower the prevalence of UA patients, but will also change the characteristics and outcomes of both the RA and UA patients.

Another important development in classifying UA patients was the coming of prediction models for the individual UA patient. Two important prediction models to predict disease outcome in UA patients are the 'Van der Helm prediction model' and the 'Visser prediction model'. These prediction models can help the Rheumatologist to decide whether or not treating the UA patient.[23,24]

PREDICTING DISEASE OUTCOME OF RA

When a patient is classified as RA, the disease course is not yet known, due to a lot of variation in the disease course of RA patients. Some patients will rapidly progress in a very erosive and disabling disease, while others will develop (spontaneous) remission. It can be very useful to know the disease course of a patient, not only to inform the patient about his prognosis but also whether or not to start (more aggressive) treatment. This is very important to avoid undertreatment, because we know that early treatment will have a positive effect on the disease course. Nevertheless, overtreatment can be even that important, as a lot of the treatments have their (toxic) side effects.

Radiographic outcome

To measure the severity of the disease, an outcome measure has to be defined. Severe RA is not uniformly defined. Patients often refer to the degree of pain or fatigue and the ability to perform daily activities and work. Rheumatologists are more concerned with the level of inflammation (expressed by the number of inflamed joints), the level of acute phase reactants, and pooled severity indices such as disease activity scores. Scientists focus on outcome measures that can be assessed objectively, such as joint destruction and mortality. These perspectives are essentially similar, since levels of impaired functionality, inflammation, and structural damage are partly correlated.[25]

Furthermore, radiographic progression is highly variable between RA patients and correlates with the cumulative burden of inflammation over time, is highly linked with physical function and other outcomes such as work disability, and is inexpensive to measure using validated scoring methods; consequently, the rate of radiographic progression is a comprehensive endpoint in observational studies.

The most common and in RA research field widely accepted measure for radiographic joint destruction of hands and feet is the Sharp-van der Heijde score. This is a quantitative score that can be used by trained readers, and comprises an erosive and narrowing (reflection of cartilage damage) score of both hands and feet with a range of 0-448.[26]

Risk factors for a more severe disease outcome

An important risk factor for RA development is to have a relative with RA. This suggests that genetic background is of importance in RA and therefore the severity of the disease course could also have a genetic background. Twin studies have indicated that the radiographic progression rate is in part heritable.[27] Recent estimations on an Icelandic RA population yielded a heritability of 45-58%.[28] Over 40 genetic variants have been identified in RA susceptibility, by genome-wide association studies (GWAS), evaluating thousands of cases and controls. These susceptibility genes are prone to be of importance in RA severity, measured by radiographic progression. Another, approach of unravelling the genetic variants for radiographic progression, are more dedicated candidate gene studies focusing on pathways important in RA, such as genes involved in inflammation and bone cartilage pathways.

The presence or high levels of several serological markers are also predictors for radiographic progression in RA. Serological markers that are important for radiographic progression are auto-antibodies and other markers related to inflammation and bone and cartilage destruction, like RF, ACPA, anti-CarP , ESR, CRP, MMP3, CTX-I, CTX-II, COMP, TIMP1, PYD, RANKL/OPG and CXCL13.[29-52]

In addition, some studies investigated whether environmental risk factors, like smoking, could be of importance in risk predicting of radiographic progression in RA.[53-55]

MRI

MRI is becoming an important tool in RA research. MRI has important advantages over conventional radiographs as, in addition to structural damage, inflammation of the synovium of the joint, tendons and bone marrow edema (BME) can be visualized and quantified. MRI produces images by detecting signal from H+ ions. The detection of H+ ions by MRI means that tissues with high concentrations of water reflect a high signal on T2-weighted sequences. This can be used to detect free fluid and inflammation. In RA this means detection of synovitis, tenosynovitis, synovial effusion and bone marrow edema. When making sequences after infusion of gadolinium containing contrast a region can be specified as an active inflammation when there is enhanced vascularity, which could distinguish between synovitis and synovial effusion.

In order to evaluate MR images an international Outcome Measures in Rheumatology Clinical Trials (OMERACT) MRI in RA working group developed and validated a semi-quantitative scoring system; the RA MRI scoring system (RAMRIS). This scoring system was developed to assess RA inflammation and damage. The scoring system recommends having at least the following sequences: imaging in two planes with T1-weighted images before and after intravenous gadolinium contrast and a T2-weighted fat saturated sequence. This scoring system measures the following RA joint pathologies; synovitis, erosion and BME.[56-

[58] Haavardsholm et al described the additional scoring of tenosynovitis.[59] The sum score ranges in unilateral MCP2-5, wrist and MTP1-5 for synovitis 0-36, erosion 0-330, BME 0-99 and tenosynovitis 0-84.

Especially BME is of special interest in RA research. Normal bone marrow is a fatty-tissue inside the bone and is visualized as a bright signal on T1-weighted images and as a dark signal on T2-weighted images. When bone marrow edema is present the fatty tissue is replaced by more water-rich material and will be visualized as a dark signal on T1-weighted images and as a bright signal on T2-weighted images.[60] Previous studies have proven by histology of a pre-surgical defined region with bone marrow edema, that these regions reveal a lymphoplasmacytic inflammatory infiltrate within trabecular bone.[61-63] Several studies showed that BME has an additive value to known diagnostic criteria, to give a higher diagnostic accuracy.[64-66] Furthermore, BME is a very strong independent predictor of radiographic progression.[67-72]

In addition, because BME has a diagnostic value for predicting RA and has a prognostic value for predicting radiographic progression, MRI detected inflammation in RA is likely to become more important in the earlier phases of RA and also the pre-clinical phase without the presence of arthritis. Recent studies showed that MRI-detected subclinical inflammation is present in patients that are in clinical remission.[73-77]

AIMS AND OUTLINE THESIS

- Identifying predictive factors for RA development.
- Identifying predictive factors for more destructive outcome of RA.

EAC cohort

The major part of this thesis was investigated in the early arthritis clinic (EAC) in Leiden. This is an ongoing observational cohort that started in 1993. Patients presenting at the outpatient clinic at the Rheumatology department at the Leiden University Medical Centre, with at least one confirmed swollen joint and symptoms less than two years were included. At baseline, patients and Rheumatologist completed questionnaires, physical examinations were performed, radiographs were taken and blood was obtained to measure acute phase reactants, RF and ACPA. From 2010 on also MRI's were taken. After two weeks, when blood and radiographs results were known, a rheumatologist diagnosed the patients as having RA, UA or another diagnosis. Thereafter yearly visits with radiographs followed.[5]

Part I is focused on unclassified arthritis and the risk on development of rheumatoid arthritis. In 2010 new criteria were developed to classify also the early RA patient. Some studies thereafter showed what the new group of RA looked like in characteristics and

outcome. The most important findings were that the group of RA, according to the 2010 criteria, were more heterogeneous and less severe in presentation and outcome.[4,6,20] In **chapter two** we determined the characteristics and outcomes of unclassified arthritis, when applying the 2010 and 1987 criteria for RA. We compared these two groups on baseline characteristics and outcomes like; DMARD initiation in the first year, fulfilling the 1987 criteria for RA during the first year and achieving remission.

In **chapter three** we analyzed UA patients according to the 2010 criteria from our own Leiden EAC cohort and compared them to UA patients from two other cohorts. We investigated the risk of development of RA in this group of patients and we tried to explorer whether the Leiden prediction rule or ACPA positivity could help identify these UA patients that progress to RA.

Part II is focused on serologic and genetic factors in predicting the severity of joint destruction in rheumatoid arthritis. When a patient can be classified as RA, nothing is known about the prognosis yet. There is a lot of variability in the disease course of RA. Several risk factors for a more severe disease course are already known, such as ACPA and several genetic variants. In **chapter four** the thus far known risk factors (or biomarkers) for a severe disease course in RA, measured by radiographic progression, are summarized. In **chapter five** genetic variants in IL-15 and the association with severity of joint damage in RA was investigated in four cohorts. In **chapter six** we investigated whether genetic variants in the IL-4 and IL-4 receptor genes were associated with the severity of joint damage in RA, in 7 cohorts. Furthermore, in **chapter seven** we investigated genetic variants in granzyme-B and the association with severity of joint damage in RA, in four cohorts.

In **chapter eight** the collagen crosslink in cartilage and bone, pyridinoline, was analyzed in relation to the severity of joint destruction in RA. We investigated whether the levels of pyridinoline on baseline and during follow-up were associated to radiographic progression in RA patients.

Part III is focused on MRI in patients with arthralgia and early arthritis. Last decades, MRI is becoming a relevant tool in RA research. MRI can be used in different stages of the disease; arthralgia, early arthritis, early RA, RA and for different purposes; for better insight in disease pathogenesis in the pre-clinical or clinical stage, or for predicting diagnosis or prognosis. In **chapter nine** we evaluated MRI inflammation in hand and foot joints in ACPA positive arthralgia patients, by comparing these joints to joints of healthy controls and ACPA positive RA patients. This pilot study was to investigate MRI pathologies in an early phase of the disease; in arthralgic joints, without clinical arthritis. In **chapter ten** we determined the concordance between inflammation at physical examination and on MRI in early arthritis patients. These two chapters give a better insight on the pathogenesis in the early clinical and clinical phase. In **chapter eleven** the relevance of the MRI detected

subclinical inflammation was investigated. We calculated the relative risk of radiographic progression in joints with MRI detected subclinical inflammation compared to that in joints without MRI detected subclinical inflammation. In **chapter twelve** we analyzed the differentiating capacity of MRI for diagnosing RA in a group of early arthritis patients.

Part IV is focused on revisiting of the MRI scan protocol. The OMERACT group developed the semi-quantitative scoring method for scoring MRI in RA; the RAMRIS method. This method also describes the scanning protocol that is needed to have the necessary MRI sequences to score the images according to this protocol. The protocol recommends to make T1 images, primarily to score erosions, a T2 or STIR sequence to score BME and a T1 post-contrast sequence to score synovitis and tenosynovitis, for every joint. This resulted in a long scanning time which is costly and in which a patient needs to keep the hand or foot motionless or otherwise will lead to artefacts. To improve this, we tried to shorten the scanning protocol, with the same accuracy and validity of scoring. First, we compared in **chapter thirteen** scoring of BME on T2 and on T1 post-contrast sequences, which could result in removing the T2 sequences from the scanning protocol. And in **chapter fourteen** we compared scoring of synovitis on T1 post-contrast and on T2 sequences, which could result in removing the T1 post-contrast from the scanning protocol and no contrast infusion would be needed.

MRI is a very sensitive tool to measure inflammation and destruction and therefore could result in a reasonable percentage of false-positives, especially when normal daily activities could influence MRI measured pathologies in RA. In **chapter fifteen** we investigated whether wearing of high heels can influence MRI results.

Finally, in **chapter sixteen** the findings of this thesis are summarized and discussed.

REFERENCE LIST

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet* 2010 Sep 25;376(9746):1094-108.
2. Schett G, Firestein GS. Mr Outside and Mr Inside: classic and alternative views on the pathogenesis of rheumatoid arthritis. *Ann Rheum Dis* 2010 May;69(5):787-9.
3. Raza K, Breese M, Nightingale P, et al. Predictive value of antibodies to cyclic citrullinated peptide in patients with very early inflammatory arthritis. *J Rheumatol* 2005 Feb;32(2):231-8.
4. Cader MZ, Filer A, Hazlehurst J, et al. Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort. *Ann Rheum Dis* 2011 Jun;70(6):949-55.
5. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011 Jan;50(1):93-100.
6. Britsemmer K, Ursum J, Gerritsen M, et al. Validation of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: slight improvement over the 1987 ACR criteria. *Ann Rheum Dis* 2011 Aug;70(8):1468-70.
7. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;7(5):R949-R958.
8. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004 Feb;50(2):380-6.
9. Finckh A, Liang MH, van Herckenrode CM, et al. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: A meta-analysis. *Arthritis Rheum* 2006 Dec 15;55(6):864-72.
10. Nell VP, Machold KP, Eberl G, et al. Benefit of very early referral and very early therapy with disease-modifying anti-rheumatic drugs in patients with early rheumatoid arthritis. *Rheumatology (Oxford)* 2004 Jul;43(7):906-14.
11. van der Linden MP, le Cessie S, Raza K, et al. Long-term impact of delay in assessment of patients with early arthritis. *Arthritis Rheum* 2010 Dec;62(12):3537-46.
12. Gerlag DM, Raza K, van Baarsen LG, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis* 2012 May;71(5):638-41.
13. Hulsemann JL, Zeidler H. Undifferentiated arthritis in an early synovitis out-patient clinic. *Clin Exp Rheumatol* 1995 Jan;13(1):37-43.
14. Huizinga TW, van der Helm-van Mil. A quantitative approach to early rheumatoid arthritis. *Bull NYU Hosp Jt Dis* 2011;69(2):116-21.
15. van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009 Aug;60(8):2262-71.
16. van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. Ann Rheum Dis 2012 Nov 28.
17. van de Stadt LA, van der Horst AR, de Koning MH, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. *Ann Rheum Dis* 2011 Jan;70(1):128-33.
18. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988 Mar;31(3):315-24.
19. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010 Sep;69(9):1580-8.

20. de Hair MJ, Lehmann KA, van de Sande MG, et al. The clinical picture of rheumatoid arthritis according to the 2010 American College of Rheumatology/European League Against Rheumatism criteria: is this still the same disease? *Arthritis Rheum* 2012 Feb;64(2):389-93.
21. Radner H, Neogi T, Smolen JS, et al. Performance of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2013 Apr 16.
22. van der Linden MP, Knevel R, Huizinga TW, et al. Classification of rheumatoid arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American College of Rheumatology/European League Against Rheumatism criteria. *Arthritis Rheum* 2011 Jan;63(1):37-42.
23. van der Helm-van Mil AH, le Cessie S, van Dongen H, et al. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007 Feb;56(2):433-40.
24. Visser H, le Cessie S, Vos K, et al. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. *Arthritis Rheum* 2002 Feb;46(2):357-65.
25. Breedveld FC, Han C, Bala M, et al. Association between baseline radiographic damage and improvement in physical function after treatment of patients with rheumatoid arthritis. *Ann Rheum Dis* 2005 Jan;64(1):52-5.
26. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000 Jan;27(1):261-3.
27. van der Helm-van Mil AH, Kern M, Gregersen PK, et al. Variation in radiologic joint destruction in rheumatoid arthritis differs between monozygotic and dizygotic twins and pairs of unrelated patients. *Arthritis Rheum* 2006 Jun;54(6):2028-30.
28. Knevel R, Grondal G, Huizinga TW, et al. Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. *Ann Rheum Dis* 2012 May;71(5):707-9.
29. Bukhari M, Thomson W, Naseem H, et al. The performance of anti-cyclic citrullinated peptide antibodies in predicting the severity of radiologic damage in inflammatory polyarthritis: results from the Norfolk Arthritis Register. *Arthritis Rheum* 2007 Sep;56(9):2929-35.
30. Courvoisier N, Dougados M, Cantagrel A, et al. Prognostic factors of 10-year radiographic outcome in early rheumatoid arthritis: a prospective study. *Arthritis Res Ther* 2008;10(5):R106.
31. Drossaers-Bakker KW, Zwinderman AH, Vliet Vlieland TP, et al. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. *Arthritis Rheum* 2002 Aug;47(4):383-90.
32. Forslind K, Ahlmen M, Eberhardt K, et al. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 2004 Sep;63(9):1090-5.
33. Kaltenhauser S, Wagner U, Schuster E, et al. Immunogenetic markers and seropositivity predict radiological progression in early rheumatoid arthritis independent of disease activity. *J Rheumatol* 2001 Apr;28(4):735-44.
34. Lindqvist E, Eberhardt K, Bendtzen K, et al. Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2005 Feb;64(2):196-201.
35. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. *Arthritis Res Ther* 2006;8(4):R128.
36. Syversen SW, Gaarder PI, Goll GL, et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 2008 Feb;67(2):212-7.
37. Vencovsky J, Machacek S, Sedova L, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. *Ann Rheum Dis* 2003 May;62(5):427-30.

38. den Broeder AA, Joosten LA, Saxne T, et al. Long term anti-tumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis* 2002 Apr;61(4):311-8.
39. Garnero P, Gineyts E, Christgau S, et al. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. *Arthritis Rheum* 2002 Jan;46(1):21-30.
40. Garnero P, Landewe R, Boers M, et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. *Arthritis Rheum* 2002 Nov;46(11):2847-56.
41. Geusens PP, Landewe RB, Garnero P, et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006 Jun;54(6):1772-7.
42. Jansen LM, van der Horst-Bruinsma I, Lems WF, et al. Serological bone markers and joint damage in early polyarthritis. *J Rheumatol* 2004 Aug;31(8):1491-6.
43. Jensen T, Klarlund M, Hansen M, et al. Connective tissue metabolism in patients with unclassified polyarthritis and early rheumatoid arthritis. Relationship to disease activity, bone mineral density, and radiographic outcome. *J Rheumatol* 2004 Sep;31(9):1698-708.
44. Knevel R, de Rooy DP, Zhernakova A, et al. Association of variants in IL2RA with progression of joint destruction in Rheumatoid Arthritis. *Arthritis Rheum* 2013 Mar 25.
45. Knevel R, van Nies JA, le Cessie S, et al. Evaluation of the contribution of cumulative levels of inflammation to the variance in joint destruction in rheumatoid arthritis. *Ann Rheum Dis* 2013 Feb;72(2):307-8.
46. Krabben A, Knevel R, Huizinga TW et al. Serum pyridinoline levels and prediction of severity of joint destruction in rheumatoid arthritis. *J Rheumatol.* In press 2013.
47. Le Loët X, Brazier M, Mejjad O, et al. Serum IgA rheumatoid factor and pyridinoline in very early arthritis as predictors of erosion(s) at two years: a simple model of prediction from a conservatively treated community-based inception cohort. *Arthritis Care Res (Hoboken)* 2010 Dec;62(12):1739-47.
48. Meeuwisse CM, van der Linden MP, Rullmann TA, et al. Identification of CXCL13 as a marker for rheumatoid arthritis outcome using an in silico model of the rheumatic joint. *Arthritis Rheum* 2011 May;63(5):1265-73.
49. Roux-Lombard P, Eberhardt K, Saxne T, et al. Cytokines, metalloproteinases, their inhibitors and cartilage oligomeric matrix protein: relationship to radiological progression and inflammation in early rheumatoid arthritis. A prospective 5-year study. *Rheumatology (Oxford)* 2001 May;40(5):544-51.
50. Skoumal M, Kolarz G, Woloszczuk W, et al. Serum osteoprotegerin but not receptor activator of NF-kappaB ligand correlates with Larsen score in rheumatoid arthritis. *Ann Rheum Dis* 2004 Feb;63(2):216-7.
51. van Tuyl LH, Voskuyl AE, Boers M, et al. Baseline RANKL:OPG ratio and markers of bone and cartilage degradation predict annual radiological progression over 11 years in rheumatoid arthritis. *Ann Rheum Dis* 2010 Sep;69(9):1623-8.
52. Young-Min S, Cawston T, Marshall N, et al. Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. *Arthritis Rheum* 2007 Oct;56(10):3236-47.
53. 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 Jun;47(6):849-54.
54. Manfredsdottir VF, Vikingsdottir T, Jonsson T, et al. The effects of tobacco smoking and rheumatoid factor seropositivity on disease activity and joint damage in early rheumatoid arthritis.

Rheumatology (Oxford) 2006 Jun;45(6):734-40.

55. Finckh A, Dehler S, Costenbader KH, et al. Cigarette smoking and radiographic progression in rheumatoid arthritis. *Ann Rheum Dis* 2007 Aug;66(8):1066-71.
56. Conaghan P, Bird P, Ejbjerg B, et al. The EULAR-OMERACT rheumatoid arthritis MRI reference image atlas: the metacarpophalangeal joints. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i11-i21.
57. Ejbjerg B, McQueen F, Lassere M, et al. The EULAR-OMERACT rheumatoid arthritis MRI reference image atlas: the wrist joint. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i23-i47.
58. Ostergaard M, Edmonds J, McQueen F, et al. An introduction to the EULAR-OMERACT rheumatoid arthritis MRI reference image atlas. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i3-i7.
59. Haavardsholm EA, Ostergaard M, Ejbjerg BJ, et al. Introduction of a novel magnetic resonance imaging tenosynovitis score for rheumatoid arthritis: reliability in a multireader longitudinal study. *Ann Rheum Dis* 2007 Sep;66(9):1216-20.
60. McQueen FM. Imaging in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2013 Aug;27(4):499-522.
61. Dalbeth N, Smith T, Gray S, et al. Cellular characterisation of magnetic resonance imaging bone oedema in rheumatoid arthritis; implications for pathogenesis of erosive disease. *Ann Rheum Dis* 2009 Feb;68(2):279-82.
62. Jimenez-Boj E, Nobauer-Huhmann I, Hanslik-Schnabel B, et al. Bone erosions and bone marrow edema as defined by magnetic resonance imaging reflect true bone marrow inflammation in rheumatoid arthritis. *Arthritis Rheum* 2007 Apr;56(4):1118-24.
63. McQueen FM, Gao A, Ostergaard M, et al. High-grade MRI bone oedema is common within the surgical field in rheumatoid arthritis patients undergoing joint replacement and is associated with osteitis in subchondral bone. *Ann Rheum Dis* 2007 Dec;66(12):1581-7.
64. Duer-Jensen A, Horslev-Petersen K, Hetland ML, et al. Bone edema on magnetic resonance imaging is an independent predictor of rheumatoid arthritis development in patients with early undifferentiated arthritis. *Arthritis Rheum* 2011 Aug;63(8):2192-202.
65. Narvaez J, Sirvent E, Narvaez JA, et al. Usefulness of magnetic resonance imaging of the hand versus anticyclic citrullinated peptide antibody testing to confirm the diagnosis of clinically suspected early rheumatoid arthritis in the absence of rheumatoid factor and radiographic erosions. *Semin Arthritis Rheum* 2008 Oct;38(2):101-9.
66. Tamai M, Kawakami A, Uetani M, et al. A prediction rule for disease outcome in patients with undifferentiated arthritis using magnetic resonance imaging of the wrists and finger joints and serologic autoantibodies. *Arthritis Rheum* 2009 Jun 15;61(6):772-8.
67. Boyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011 Mar;70(3):428-33.
68. Haavardsholm EA, Boyesen P, Ostergaard M, et al. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. *Ann Rheum Dis* 2008 Jun;67(6):794-800.
69. Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009 Mar;68(3):384-90.
70. Hetland ML, Stengaard-Pedersen K, Junker P, et al. Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 2010 Oct;69(10):1789-95.
71. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 1999 Mar;58(3):156-63.

72. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003 Jul;48(7):1814-27.
73. Brown AK, Quinn MA, Karim Z, et al. Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression. *Arthritis Rheum* 2006 Dec;54(12):3761-73.
74. Brown AK, Conaghan PG, Karim Z, et al. An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* 2008 Oct;58(10):2958-67.
75. Gandjbakhch F, Foltz V, Mallet A, et al. Bone marrow oedema predicts structural progression in a 1-year follow-up of 85 patients with RA in remission or with low disease activity with low-field MRI. *Ann Rheum Dis* 2011 Dec;70(12):2159-62.
76. Gandjbakhch F, Conaghan PG, Ejbjerg B, et al. Synovitis and osteitis are very frequent in rheumatoid arthritis clinical remission: results from an MRI study of 294 patients in clinical remission or low disease activity state. *J Rheumatol* 2011 Sep;38(9):2039-44.
77. Gandjbakhch F, Haavardsholm EA, Conaghan PG, et al. Determining a Magnetic Resonance Imaging Inflammatory Activity Acceptable State Without Subsequent Radiographic Progression in Rheumatoid Arthritis: Results from a Followup MRI Study of 254 Patients in Clinical Remission or Low Disease Activity. *J Rheumatol* 2013 Dec 15.

PART 1

Unclassified arthritis and the risk on development of rheumatoid arthritis

2

Undifferentiated arthritis characteristics and outcomes when applying the 2010 and 1987 criteria for rheumatoid arthritis

A. Krabben, T. W. J. Huizinga, A. H. M. van der Helm-van Mil

Annals of the Rheumatic Diseases, 2012 February;71(2):238–241.

ABSTRACT

Objective

Undifferentiated arthritis (UA) is a diagnosis 'per exclusionem'. Therefore this patient population may change since the development of the ACR/EULAR 2010-criteria for RA. This study evaluated characteristics and outcomes of UA in its new shape. Second, it was evaluated whether the 2010-criteria and the Leiden prediction rule were congruent in categorizing UA-patients.

Methods

2,472 early arthritis patients were studied. RA was classified according to either the 1987 or the 2010-criteria. UA was defined as not fulfilling existing classification criteria. UA-patients were compared for baseline characteristics and outcomes. In 1987-UA- patients both the 2010-criteria and the Leiden prediction rule were applied and categorization compared.

Results

2010-UA-patients (n=776) had milder baseline characteristics than 1987-UA-patients (n=1,166). During follow-up, still 24% of the 2010-UA-patients fulfilled the 1987 RA-criteria compared to 32% of the 1987-UA-patients. The 2010-UA-patients started less frequent DMARD- therapy and reached more frequent sustained DMARD-free remission. 30% of 2010-criteria-positive patients were predicted to have a low risk on RA; these patients achieved more frequent DMARD-free sustained remission than other 2010-criteria-positive patients.

Conclusion

UA in the era of the 2010-criteria is less prevalent and milder at presentation and in outcome. This implies that UA-patients with unfavorable characteristics are now more often classified as RA.

INTRODUCTION

A considerable proportion of the patients presenting with synovitis of recent onset have undifferentiated arthritis. Undifferentiated arthritis is identified when none of the existing classification criteria for definitive diagnoses are fulfilled and arthritis is not septic or caused by crystals. The 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for rheumatoid arthritis (RA) were recently developed.[1] Recent studies have indicated that these criteria are fulfilled earlier in the disease and more frequently than with the 1987 ACR criteria.[2,3] Since undifferentiated arthritis is a disease 'per exclusionem', we questioned how this disease entity is characterized when applying the 2010 criteria. Therefore, the first aim of this study was to explore the characteristics and the outcome of undifferentiated arthritis in its new form. Before the 2010 criteria were formulated, a prediction rule was developed that aimed to determine individual undifferentiated arthritis patient's chances of developing RA (according to the 1987 criteria).[4] Both methods are particularly applicable to patients who could not be diagnosed otherwise, thus to former undifferentiated arthritis patients. We also evaluated whether the 2010 criteria and the prediction rule were congruent in categorizing early undifferentiated arthritis patients.

PATIENTS AND METHODS

Patients

Two thousand four hundred and seventy-two early arthritis patients, included in the Leiden Early Arthritis Clinic (EAC) between 1993 and February 2010 and who had a follow-up of at least 1 year, were studied. The EAC is a population-based prospective cohort that includes patients with confirmed arthritis and symptoms for less than 2 years.[5] At baseline, patients and rheumatologists completed questionnaires, physical examinations (including 68 tender and 66 swollen joint counts) were performed, radiographs were taken, and blood was obtained for determination of the C-reactive protein level, erythrocyte sedimentation level, IgM rheumatoid factor and anti-citrullinated protein antibodies. After 2 weeks, when blood and radiographic results were known, a rheumatologist diagnosed the patients as having RA, undifferentiated arthritis, or another diagnosis; this was done using the 1987 criteria for RA. This resulted in 1166 patients with undifferentiated arthritis (the so-called 1987 undifferentiated arthritis patients). We applied the 2010 criteria to the same patients and subsequently identified the so-called 2010 undifferentiated arthritis patients (n=776). Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee.

Outcome

Follow-up visits were performed annually. After 1 year it was evaluated whether the 1987 criteria were fulfilled and whether disease-modifying anti-rheumatic drugs (DMARD) were initiated. Patients treated according to a randomized trial were not studied (n=145) for this outcome. The prescribed DMARD in RA patients differed in time; hydroxy- choroquine, penicillamine, or sulfasalazine were the initial DMARD in the 1990s, and methotrexate was the initial DMARD since 1999. During a follow-up of 7 years, it was evaluated whether a patient achieved sustained remission. Sustained remission was defined as the absence of synovitis for at least 1 year after the cessation of eventual DMARD therapy.[6] Furthermore, the functional ability was measured annually by the health assessment questionnaire (HAQ) score. The difference in the HAQ score over 7 years was also evaluated.

Comparison of categorization

In order to determine whether classification according to the 2010 criteria was congruent with risk estimation by the prediction rule, both methods were applied to 1,162 1987 undifferentiated arthritis patients. The congruency of classification was evaluated, as well as the disease outcome. The primary outcome here was having persistent disease (absence of sustained DMARD-free remission).

Analysis

Patient characteristics were compared using the χ^2 test for nominal variables, the Student's t test for continuous variables and the Mann-Whitney U-test for non-parametric testing. Analyses of sustained DMARD-free remission were performed using univariate Cox regression analyses. The difference in HAQ score between 1987 undifferentiated arthritis patients and 2010 undifferentiated arthritis patients was compared using a multivariate normal regression analysis. As the HAQ changed over time, the interaction of the undifferentiated arthritis groups with time was entered into the model as categorical variables. p Values ≤ 0.05 were considered significant. Analyses were done using SPSS, version 17.0.

RESULTS

Characteristics at presentation

Among all 2472 early arthritis patients, 772 (31.2%) patients were diagnosed other than undifferentiated arthritis or RA. Using the 1987 criteria for RA, 534 (21.6%) patients had RA and 1166 (47.2%) patients had undifferentiated arthritis. In four patients only one serological test and one acute-phase response measure was obtained and therefore they could not be classified according to the 2010 criteria.[1] Subsequently, the 2010 criteria were applied to 1696 undifferentiated arthritis and RA patients. Now 920 (54.2%) patients were

Table 1. Patient characteristics of 1987-UA-patients and 2010-UA-patients, at first presentation

Characteristic	1987 UA (n = 1,166)	2010 UA (n = 776)	*P*
Age, mean ± SD in years	51.6 ± 17.1	50.3 ± 17.2	0.12
Female sex	699 (59.9)	443 (57.1)	0.21
BMI, mean ± SD in kg/m^2	25.9 ± 4.3	25.6 ± 4.4	0.26
Current or past smoker	286 (28.0)	197 (29.1)	0.97
Onset of complaints			
(Sub)acute	657 (58.8)	465 (62.4)	0.12
Gradual	460 (41.2)	280 (37.6)	
Morning stiffness, median (IQR) in minutes	30 (0-60)	30 (0-60)	0.06
0-29	457 (41.0)	337 (45.6)	0.14
30-59	190 (17.0)	117 (15.8)	
≥60	468 (42.0)	285 (38.6)	
Symptom duration, median (IQR) in weeks	15.7 (6.9-31.9)	13.4 (5.3-29.2)	0.01
Localization of affected joints			
Small joints hands/feet	908 (81.1)	631 (84.8)	0.04
Localization of affected joints			
Symmetrical	563 (54.0)	328 (47.7)	0.01
Localization of affected joints			
Upper extremities	722 (72.1)	507 (75.7)	0.10
Swollen joint count, median (IQR)	3 (1-7)	2 (1-5)	<0.001
0-3 joints	607 (53.1)	494 (65.3)	<0.001
4-10 joints	364 (31.8)	218 (28.8)	
>10 joints	172 (15.0)	44 (5.8)	
Tender joint count, median (IQR)	5 (2-12)	2 (1-6)	<0.001
0-3 joints	301 (40.2)	266 (59.2)	<0.001
4-10 joints	223 (29.8)	158 (35.2)	
>10 joints	225 (30.0)	25 (5.6)	
ESR, median (IQR) mm/hour	19 (8-39)	16 (8-36)	0.02
CRP, median (IQR) mg/liter	9 (3-24.5)	8 (3-23)	0.29
RF positive	294 (25.5)	76 (9.9)	<0.001
Low positive	118 (10.2)	32 (4.2)	0.83
High positive	176 (15.3)	44 (5.7)	
Anti-CCP-2 positive	234 (24.1)	61 (9.6)	<0.001
Low positive	44 (4.5)	44 (6.9)	<0.001
High positive	190 (19.6)	17 (2.7)	
HAQ, mean ± SD	0.8 ± 0.7	0.7 ± 0.6	0.01
2010 criteria score, mean ± SD	4.9 (2.4)	3.2 (1.4)	<0.001

* Except where indicated otherwise, values are the number (%) of patients.

IQR = interquartile range.

Localization of affected joint: 'upper joints' stands for upper joints with or without involvement of the lower joints, whereas 'small joints' stands for smaller joints with or without involvement of the large joints.

In the group of all patients with UA, some data were missing, as follows: for BMI, n=351; for smoking, n=146; for onset of complaints, n=48; for morning stiffness, n=51; for symptom duration, n=95; for small joints involved, n=46, for symmetrical joint involvement, n=123; for upper extremity joints involved, n=164; n=25; for ESR, n=8; for CRP, n=81; for RF, n=11; for CCP, n=197; for HAQ score, n=245. A chi-square test was used for nominal variables and the Student's t-test or Mann-Whitney U-test for continuous variables. Student's t-test was performed when variables are presented as mean and a Mann-Whitney U-test was performed when variables are presented as median.

classified as having RA; therefore 776 (45.8%) 2010 undifferentiated arthritis patients were identified.

The baseline characteristics of the 2010 undifferentiated arthritis patients and 1987 undifferentiated arthritis patients were compared (table 1). Overall, 2010 undifferentiated arthritis patients had milder disease characteristics than the 1987 undifferentiated arthritis patients.

Outcome

After 1 year of follow-up, 23.8% of the 2010 undifferentiated arthritis patients fulfilled the 1987 criteria, compared with 32.2% of the 1987 undifferentiated arthritis patients (p=0.001). Likewise, in 32.7% of the 2010 undifferentiated arthritis patients a DMARD was initiated during the first year compared with 45.0% of the 1987 undifferentiated arthritis patients (p<0.001).

During 7 years of follow-up, the 2010 undifferentiated arthritis patients achieved sustained remission more often (45.9%) than the 1987 undifferentiated arthritis patients (34.2%) (HR 1.45, 95% CI 1.20 to 1.75; figure 1). This implies that more than half of the 2010 undifferentiated arthritis patients have persistent disease. The baseline characteristics of these patients are presented in supplementary table S1, available online only. The HAQ scores were not significantly different between the two undifferentiated arthritis groups during 7 years of follow-up (p=0.92).

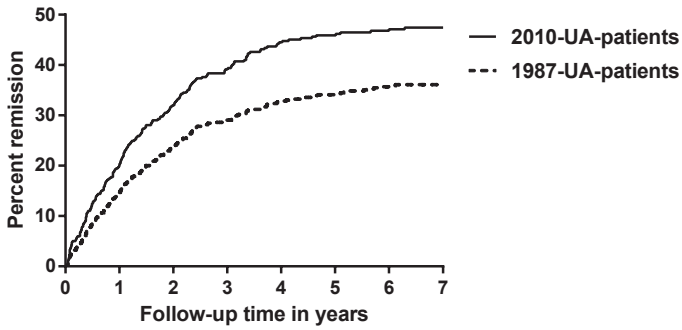


Figure 1. Kaplan-Meier curve of the percentage of patients with sustained disease-modifying antirheumatic drug (DMARD)-free remission in 1987-UA-patients compared to 2010-UA-patients.

Comparison of categorization

All 1,162 1987 undifferentiated arthritis patients were classified according to the 2010 criteria and the prediction rule (table 2). Only 0.6% of all undifferentiated arthritis patients were classified as 2010 criteria-negative but were predicted to have a high risk of RA. In contrast, 30.3% (148/489) of the 2010 criteria- positive patients were in the low risk of RA

Table 2. Comparison of categorization, according to the prediction model and the 2010 criteria, applied to 1987-UA-patients and outcome of these patients over time.

	2010 criteria negative	2010 criteria positive	Total
	593 (51.0%)	148 (12.7%)	
Low risk on RA score ≤6	*Outcome* -Persistent disease 49.1% -Initiation DMARD 29.3% -Fulfilling 1987 criteria 11.6%	*Outcome* -Persistent disease 62.9% -Initiation DMARD 48.2% -Fulfilling 1987 criteria 31.1%	741 (63.7%)
	73 (6.3%)	227 (19.5%)	
Intermediate risk on RA score >6 and ≤8	*Outcome* -Persistent disease 46.9% -Initiation DMARD 41.2% -Fulfilling 1987 criteria 35.6%	*Outcome* -Persistent disease 81.4% -Initiation DMARD 64.5% -Fulfilling 1987 criteria 63.0%	300 (25.8%)
	7 (0.6%)	114 (9.8%)	
High risk on RA score >8	*Outcome* -Persistent disease 100.0% -Initiation DMARD 0.0% -Fulfilling 1987 criteria 85.7%	*Outcome* -Persistent disease 92.0% -Initiation DMARD 77.9% -Fulfilling 1987 criteria 74.6%	121 (10.4%)
Total	673 (57.9%)	489 (42.0%)	1162 (100%)

Both the 2010 criteria and the prediction rule were applied to 1,162 UA patients, according to the 1987 criteria. Patients were categorized in the groups '2010 criteria negative' or '2010 criteria positive' and stratified in three groups according to the prediction model. Accordingly these patients were subdivided in six cells. Three outcome measures were studied in each cell of patients; disease persistency during seven years of follow-up, initiation of a DMARD in the first year of follow-up and fulfilling the 1987 criteria at one year of follow-up.

group. During follow-up 62.9% of these latter patients had persistent disease, which is a bit lower than the other 2010 criteria-positive patients (table 2).

DISCUSSION

This study aimed to characterize undifferentiated arthritis in its recent form, after new classification criteria for RA have been developed. It was observed that undifferentiated arthritis is still prevalent but less frequent compared with when the 1987 ACR criteria for

RA are applied. In addition, we observed that both characteristics at disease presentation and the disease outcome of 2010 undifferentiated arthritis patients were milder than those of the 1987 undifferentiated arthritis patients.

With regard to the implication of the changing concept of undifferentiated arthritis, differences in disease outcome are more essential than differences at disease onset. Importantly, 2010 undifferentiated arthritis patients more often achieved sustained DMARD-free remission than 1987 undifferentiated arthritis patients. This implies that undifferentiated arthritis patients with unfavorable characteristics are now more often classified as having RA. The finding that 23.8% of the 2010 undifferentiated arthritis patients fulfilled the 1987 criteria for RA within 1 year of follow-up suggests that although the 2010 criteria classify RA earlier than the 1987 criteria,[3] the 2010 criteria do not recognize approximately a quarter of the RA patients already at baseline.

We also evaluated whether the 2010 criteria and a previously derived prediction rule were congruent in categorizing 1987 undifferentiated arthritis patients as having (a high probability of) RA. It was observed that the majority who did not fulfil the 2010 criteria were also in the low-risk group of the prediction rule. However, one-third of the 2010 criteria-positive patients were predicted to have a low risk of RA. During the course of the disease, these latter 2010 criteria-positive patients less frequently had persistent arthritis than the other 2010 criteria-positive patients.

Interpretation of the outcome initiation of a DMARD within the first year should be done with some caution. In the 2000s DMARD were prescribed more frequently in undifferentiated arthritis than in the 1990s. Consequently, the frequency of DMARD initiation was higher in the second compared to the first time period. Nevertheless, patients belonging to the different inclusion years were equally distributed among the categories depicted in table 2, allowing comparisons between groups. In addition, a stratified analysis in patients included before and after 2000 yielded comparable results (data not shown).

A second argument that DMARD use or fulfilling the 1987 criteria are not optimal outcome measures for the present comparison is that the first was the outcome measure used when deriving the prediction rule and the latter the outcome measure used when deriving the 2010 criteria. This might result in circle effects. Disease persistence was not used while deriving either tool, and is therefore the most neutral outcome for the purpose of this comparison. Another consideration is that 213 of the presently studied patients were also used in the data-driven phase of the derivation of the 2010 criteria and 570 of the 1166 undifferentiated arthritis patients were used for the derivation of the prediction rule.

Evaluation of congruency of both methods is formally not correct, as the 2010 criteria were meant for classification and the prediction rule was derived to estimate individual patients' probability of fulfilling the 1987 criteria at an early stage. On the other hand, the 2010 criteria will most likely also be used for individual patients in the clinic. Although a comparison of the disease outcomes of the concordantly and discordantly classified pa-

tients should be considered reluctantly, an evaluation as to whether both the classification criteria and the prediction rule identify patients with RA early is clinically relevant.

In conclusion, the present data revealed that undifferentiated arthritis when applying the 2010 criteria is less prevalent and milder at presentation and in outcome. In addition, it was observed that 24% of 2010 criteria-negative patients fulfilled the 1987 criteria within 1 year. This implies that although undifferentiated arthritis patients with unfavorable characteristics are now more often classified as having RA, careful clinical observation of 2010 undifferentiated arthritis patients is indicated.

REFERENCE LIST

1. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
2. Cader MZ, Filer A, Hazlehurst J, et al. Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort. *Ann Rheum Dis.* Published Online First: 1 February 2011. doi:10.1136/ard.2010.143560.
3. van der Linden MP, Knevel R, Huizinga TW, et al. Classification of rheumatoid arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American College of Rheumatology/European League Against Rheumatism criteria. *Arthritis Rheum* 2011;63:37–42.
4. van der Helm-van Mil AH, le Cessie S, van Dongen H, et al. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007;56:433–40.
5. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes – what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011;50:93–100.
6. van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009;60:2262–71.

Supplementary table 1: Baseline characteristics of patients that at inclusion were 2010-criteria positive or 2010-criteria negative and over time had, or not had, a persistent disease

Characteristic	2010 criteria negative, without persistent disease (n = 182)	2010 criteria negative, with persistent disease (n = 197)	2010 criteria positive (n=337)	P#
Morning stiffness, median (IQR) in minutes	12.5 (0-60)	17.5 (0-60)	60 (15-120)	<0.001
Swollen joint count (66 joint count), median (IQR)	1 (1-4)	2 (1-4)	7 (3-12)	<0.001
Tender joint count, median (IQR)	1 (1-3.3)	2 (1-5)	12 (6-20)	<0.001
ESR, median (IQR) mm/hour	15 (7-75)	18 (8-37)	28 (12-46)	<0.001
CRP, median (IQR) mg/liter	7 (3-21)	9 (3-22.8)	11 (5-28)	0.061
RF positive	7 (3.8)	11 (5.6)	183 (54.5)	<0.001
Anti-CCP-2 positive	3 (1.9)	9 (4.9)	167 (50.6)	<0.001
HAQ, mean ± SD	0.6 ± 0.6	0.7 ± 0.7	0.9 ± 0.7	0.002
2010 criteria score, mean ± SD	2.9 ± 1.4	3.2 ± 1.4	7.4 ± 1.2	<0.001

* Except where indicated otherwise, values are the number (%) of patients.
IQR = interquartile range.
#For this p-value the 2010-criteria-negative patients with persistent disease were compared to the 2010-criteria-positive patients.
Analysis were done on patients that had enough follow-up and data on remission and persistency of the disease.
A chi-square test was used for nominal variables and the Student's t-test or Mann-Whitney U-test for continuous variables. Student's t-test was performed when variables are presented as mean and a Mann-Whitney U-test was performed when variables are presented as median.

3

Risk of rheumatoid arthritis development in patients with unclassified arthritis according to the 2010 ACR/EULAR criteria for rheumatoid arthritis

A. Krabben, A. Abhishek, K. Britsemmer, A. Filer,
T. W. J. Huizinga, K. Raza, D. J. van Schaardenburg,
A. H. M. van der Helm-van Mil

Rheumatology (Oxford), 2013 July;52(7):1265-1270.

ABSTRACT

Objective

Early recognition and treatment of RA is associated with an improved outcome. The 2010 ACR/EULAR criteria for RA identify RA patients earlier than the 1987 ACR criteria. Nevertheless, we recently observed that 24% of the 2010 unclassified arthritis (UA) patients develop RA during follow-up. Here we studied this frequency in other cohorts and evaluated the prognostic accuracy of ACPA and the Leiden prediction rule in 2010 UA patients.

Methods

The 2010 UA patients from three Early Arthritis Clinics were studied: 776 from Leiden, 121 from Birmingham and 322 from Amsterdam. Fulfilment of the 1987 ACR criteria during follow-up was studied as the primary outcome. DMARD prescription during the year and having a persistent course of arthritis over 7 years were studied as secondary outcomes in one cohort. The presence of ACPA and the prediction score at baseline were evaluated in relation to these outcomes.

Results

In the three cohorts, 24%, 26% and 12%, respectively, of the 2010 UA patients fulfilled the 1987 criteria after 1 year. However, some of these patients already fulfilled the 1987 criteria at baseline. In 1987 and 2010 UA patients, 15%, 21% and 9%, respectively, developed RA (1987) at 1 year. In these patients, 0-6% of the patients were ACPA positive and 0-1% had high prediction scores. Consequently a large majority of the UA patients with an unfavorable outcome was not recognized by these prognostic tools.

Conclusion

A proportion of 2010 UA patients progress to RA. ACPA and the Leiden prediction rule are not useful in identifying these patients. These results imply that other predictive markers should be developed for 2010 UA patients.

INTRODUCTION

Persistence of inflammation and destruction of joints are hallmarks of RA. Several observational cohort studies and clinical trials demonstrate that early initiation of treatment is associated with more remission and less joint destruction.[1-3] In order to initiate treatment early, adequate referral strategies are required, as well as tools that identify RA patients among all patients with early unclassified arthritis (UA)[4] Several biomarkers have been identified, of which ACPAs are among the most potent. Combining several risk factors resulted in the construction of prediction models; of these the Leiden prediction rule is widely validated.[5,6] However, these studies were performed in patients with UA when RA was defined using the 1987 ACR criteria.

Recently, the 2010 ACR/EULAR criteria for RA were developed. A major reason to derive new criteria was to obtain criteria that are fulfilled at an early stage of RA.[7] Current data show that the 2010 criteria are indeed fulfilled earlier than the 1987 criteria.[8-10] Consequently the group of patients classified as UA when the 2010 criteria are applied has changed. The so-called 2010 UA patients have milder characteristics at disease onset and as a group also have a less severe disease outcome.[8,10] Nonetheless, not all patients who are eventually diagnosed with RA fulfil the 2010 criteria at first presentation. We recently observed that 24% of the early 2010 UA patients fulfilled the 1987 criteria during the first year of followup.[11] Ideally these patients should also be identified at initial presentation in order to achieve early and individualized treatment strategies.

This study aimed to evaluate (i) the proportion of 2010 UA patients who fulfil the 1987 criteria during the first year of the disease in other independent early arthritis cohorts and (ii) the prognostic value of available prognostic markers (ACPA and the Leiden prediction rule) in 2010 UA patients in all three cohorts.

PATIENTS AND METHODS

Patients

The study comprised early arthritis patients of three different cohorts. First, patients from the Leiden Early Arthritis Clinic (EAC) cohort were studied. This population-based longitudinal cohort started in 1993 and included patients with arthritis of at least one joint and symptom duration <2 years. At first visit, questionnaires were completed, physical examination performed, radiographs taken and blood obtained for determination of CRP, ESR, IgM-RF and ACPA. Follow-up visits were performed yearly.[12]

The second studied cohort comprised patients from the Birmingham very early arthritis clinic that started in 2000. Patients were included in cases of clinical synovitis of at least one joint and symptom duration (of any symptom attributed by the rheumatologist to inflam-

matory arthritis) of 43 months with no prior DMARD treatment. At baseline, demographic data, physical examination and radiographs were obtained and blood was obtained for determination of CRP, ESR, IgM-RF and ACPA. Follow-up visits were performed at 1, 2, 3, 6, 12 and 18 months.[9]

Third, early arthritis patients from the EAC at the Jan van Breemen Research Institute/ Reade in Amsterdam, The Netherlands, which started in 1995, were studied. Patients were included with at least two swollen joints, symptom duration of <2 years and no prior DMARD treatment. Patients with OA, crystal arthropathy, SpA, SLE, SS and infectious arthritis were excluded. At baseline, demographic data were collected, physical examination was performed, radiographs were taken and blood was obtained for determination of CRP, ESR, IgM-RF and ACPA. Follow-up visits were performed at 3 month intervals during the first year (Table 1).[8]

Table 1: Baseline characteristics of '2010-UA-patients' in the three cohorts

Characteristic	Leiden-EAC* (n=776)	Birmingham-EAC** (n=121)	Amsterdam-EAC*** (n=322)
Age, mean±SD, years	50.3±17.2	47.9±18.3	50.1±15.0
Female sex, n (%)	443 (57.1)	65 (53.7)	197 (61.2)
Morning stiffness, median (IQR), min	30 (0-60)	30 (0-120)	30 (8-60)
Swollen joint count (66 joint count), median (IQR)	2 (1-5)	2 (1-3)	3 (1-5)
Tender joint count (68 joint count), median (IQR)	2 (1-6)	2 (1-4)	3 (1-5)
Localisation of affected joints			
Small joints hands/feet, n (%)	492 (66.1)	92 (76.0)	135 (41.9)
Symmetrical, n (%)	328 (47.7)	20 (16.5)	243 (75.5)
Upper extremities, n (%)	468 (69.8)	75 (62.0)	98 (30.4)
CRP, median (IQR), mg/l	8 (3-23)	17 (<5-45)	7 (3-21)
ACPA positive, n (%)	61 (9.6)	1 (0.8)	15 (4.7)
RF positive, n (%)	76 (9.9)	3 (2.5)	14 (4.3)

Except where indicated otherwise, values are the number (%) of patients. IQR = interquartile range, SD = standard deviation. Localisation of affected joint: 'upper extremities' stands for upper joints with or without involvement of the lower joints, whereas 'small joints' stands for smaller joints with or without involvement of the large joints.

*In the Leiden-EAC cohort, some data were missing, as follows: for morning stiffness, n=37; for swollen joint count (SJC), n=22; for tender joint count (TJC), n=337; for small joints involved, n=32, for symmetrical joint involvement, n=89; for upper extremity joints involved, n=106; for C-reactive protein (CRP), n=58; for anti-citrullinated-peptide antibodies (ACPA), n=143; for rheumatoid factor (RF), n=10.

**In the Birmingham-EAC, some data were missing, as follows: for morning stiffness, n=1, for CRP, n=4, for ACPA, n=3, for RF, n=8.

***In the Amsterdam-EAC cohort, some data were missing, for morning stiffness, swollen joint count (SJC), tender joint count (TJC), symmetrical joint involvement and upper extremity joints involvement, n=8.

In all three cohorts, the baseline diagnosis was assessed. First, other diagnoses were excluded, such as ReA, PsA, crystal arthropathy, SpA, SLE and sarcoidosis. Subsequently, RA was classified according to the 2010 criteria where radiologic information was not taken into account.[9,10] Then the remaining patients were classified as 2010 UA and were studied here. Furthermore, we evaluated which of the 2010 UA patients fulfilled the 1987 criteria at baseline. Patients who were classified as UA according to both the 1987 and 2010 criteria (1987 and 2010 UA patients) were also studied (see supplementary Fig. S1 for an overview, available at Rheumatology Online). All three cohorts obtained written informed consent from the participants. The studies were approved by the local medical ethic committees.

Outcome

Fulfilling the 1987 criteria during the first year of follow-up was the outcome measure studied. The 1987 criteria could be fulfilled cumulatively during follow-up. Although fulfilling the 1987 criteria may not be an early phenomenon, these criteria still reflect the core phenotype of RA. Since it can be argued that this outcome is subject to some degree of circularity, two other outcomes were studied in the Leiden dataset, DMARD initiation during the first year and disease persistency over 7 years of follow-up. Patients treated according to a randomized trial were not studied for the outcome DMARD initiation during the first year. Arthritis persistency was defined as the absence of a sustained DMARD-free remission, which was described as the absence of synovitis for at least 1 year after cessation of eventual DMARD therapy.[13]

Analysis

Analyses were done both on 2010 UA patients and 1987 and 2010 UA patients using SPSS version 17.0. The proportion of 2010 UA patients and 1987 and 2010 UA patients who progressed to RA was determined. ACPA positivity and the Leiden prediction score (grouped as score 46, >6 and <8 and 58) were evaluated in relation to the development of RA. The Leiden prediction model consists of nine items. Each prediction score varied from 0 to 14 and corresponded to the percent chance of developing RA. The higher the score, the higher the chance of progressing to RA. A score of 46 is associated with a negative predictive value (NPV) of 91% and a score 58 with a positive predictive value (PPV) of 84%. The area under the receiver operator characteristic curve (AUC) was determined; the prediction score was analyzed as continuous variable.

RESULTS

In total, 1219 2010 UA patients, originating from three different early arthritis clinics, were studied: 776 from Leiden, 121 from Birmingham and 322 from Amsterdam (see supplementary Fig. S1 for an overview, available at Rheumatology Online). In these three cohorts, 24%, 26% and 12% of the 2010 UA patients, respectively, fulfilled the 1987 ACR criteria during the first year of follow-up.

As a proportion of the 2010 UA patients fulfilled the 1987 criteria at baseline (103, 7, 86 patients in the three cohorts, respectively), progression to RA was subsequently studied in the subgroup of patients who were classified as UA according to both sets of criteria. Now 673, 114 and 236 1987 and 2010 UA patients were studied, of whom 15%, 21% and 9%, respectively, progressed to RA.

Next, we evaluated the extent to which UA patients who fulfil the 1987 criteria during follow-up could be identified at baseline using ACPA or the Leiden prediction rule. First, 2010 UA patients were studied. The frequency of ACPA positivity in the different cohorts with 2010 UA patients varied between 0% and 7%. When evaluating the prediction scores, it was observed that a prediction score of 58 was seldom obtained. The AUC of ACPA ranged between 0.49 and 0.60 and that of the prediction score between 1.59 and 0.81.

Subsequently, the 1987 and 2010 UA patients were evaluated. The frequency of ACPA positivity or high prediction scores was even lower here (Table 2). This prohibited determination of PPVs and reduced the clinical utility of these markers in these patients. The NPVs of ACPA were 83%, 79% and 91%, respectively. The NPVs of prediction scores 46 were 88%, 81% and 90%, respectively. Although these NPVs seem adequate, these chances were comparable to the prior chances of not developing RA, which were 85%, 79% and 91%, respectively. The AUCs of both predictive markers ranged between 0.49 and 0.55 for the ACPA and between 0.53 and 0.78 for the prediction score (supplementary Table S1, available at Rheumatology Online).

Since we observed that the majority of 1987 and 2010 UA patients who progressed to RA within the first year of follow-up were ACPA negative and had low prediction scores, we studied the baseline characteristics of these patients in more depth. This revealed an overall milder phenotype at baseline of 1987 and 2010 UA patients who did not progress to RA compared with those who did (supplementary Table S2, available at Rheumatology Online).

The main outcome studied was fulfilling the 1987 ACR criteria after 1 year of follow-up. DMARD initiation during year 1 and disease persistency over 7 years (absence of sustained DMARD-free remission) were also assessed as secondary outcomes. During the first year, 39% of the 2010 UA patients and 34% of the 1987 and 2010 UA patients initiated a DMARD. Over the 7-year follow-up in the Leiden dataset, 54% of the 2010 UA patients

Table 2: Stratification of ACPA positivity and the Leiden prediction score in relation to the development of RA in '2010-UA-patients' as well as in '2010-UA-and-1987-UA-patients' in three different cohorts.

			Leiden EAC*						Birmingham EAC**		Amsterdam EAC***	
			RA-1987 at 1 yr		DMARD initiation at 1 yr		Persistent disease 7 yr		RA-1987 at 1 yr		RA-1987 at 1 yr	
			-	+	-	+	-	+	-	+	-	+
2010-UA	ACPA positivity	-	439 *(69%)*	133 *(21%)*	263 *(62%)*	141 *(33%)*	168 *(44%)*	200 *(52%)*	87 *(74%)*	30 *(25%)*	268 *(83%)*	39 *(12%)*
		+	18 *(3%)*	43 *(7%)*	9 *(2%)*	12 *(3%)*	4 *(1%)*	14 *(4%)*	0 *(0%)*	1 *(1%)*	14 *(4%)*	1 *(0%)*
	Prediction rule score	≤6	547 *(70%)*	110 *(14%)*	311 *(58%)*	176 *(33%)*	180 *(43%)*	206 *(49%)*	79 *(69%)*	21 *(18%)*	213 *(68%)*	28 *(9%)*
		>6 and <8	42 (5%)	49 *(6%)*	18 *(3%)*	26 *(5%)*	11 *(3%)*	17 *(4%)*	4 *(4%)*	10 *(9%)*	60 *(19%)*	11 *(4%)*
		≥8	1 *(0%)*	27 *(3%)*	1 *(0%)*	3 *(1%)*	1 *(0%)*	3 *(1%)*	0 *(0%)*	0 *(0%)*	1 *(0%)*	0 *(0%)*
2010-UA & 1987-UA	ACPA positivity	-	425 *(79%)*	84 *(16%)*	259 *(67%)*	113 *(29%)*	159 *(46%)*	176 *(51%)*	87 *(78%)*	23 *(21%)*	202 *(86%)*	20 *(8%)*
		+	17 *(3%)*	13 *(2%)*	8 *(2%)*	7 *(2%)*	3 *(1%)*	9 *(3%)*	0 *(0%)*	1 *(1%)*	13 *(6%)*	1 *(0%)*
	Prediction rule score	≤6	524 *(78%)*	69 *(10%)*	301 *(61%)*	148 *(30%)*	168 *(43%)*	187 *(48%)*	79 *(74%)*	18 *(17%)*	170 *(75%)*	18 *(8%)*
		>6 and <8	47 *(7%)*	26 *(4%)*	22 *(4%)*	21 *(4%)*	15 *(4%)*	15 *(4%)*	4 *(4%)*	6 *(6%)*	36 *(16%)*	2 *(1%)*
		≥8	1 *(0%)*	6 *(1%)*	2 *(0%)*	0 *(0%)*	0 *(0%)*	2 *(1%)*	0 *(0%)*	0 *(0%)*	1 *(0%)*	0 *(0%)*

*Leiden-EAC: 2010-UA n=776 (ACPA status n=633), 2010-UA&1987-UA n=673 (ACPA status n=539). Persistent disease: 2010-UA n=418 (ACPA status n=386), 2010-UA&1987-UA n=387 (ACPA status n=347)

**Birmingham-EAC: 2010-UA n=121 (ACPA status n=118, prediction rule n=114), 2010-UA&1987-UA n=114 (ACPA status n=111, prediction rule n=107).

***Amsterdam-EAC: 2010-UA n=322 (prediction rule n=313), 2010-UA&1987-UA n=236 (prediction rule n=227).

Except where indicated otherwise, values are the number (%) of patients.

and 53% of the 1987 and 2010 UA patients had persistent disease. However, with these outcomes the majority of patients with DMARD initiation and/or persistent arthritis were not recognized by ACPA or the prediction model, which was due to the low prevalence of ACPA and high prediction scores in these UA patients (Table 2).

DISCUSSION

This study evaluated the frequency of development of RA (1987 criteria) in 2010 UA patients in three independent cohorts and showed that a large number of 2010 UA patients fulfil the 1987 criteria during follow-up. Second, it was evaluated whether ACPA or a previously derived prediction rule is useful in identifying which 2010 UA patients will progress to RA (1987). Analyses on 2010 UA patients from three clinics consistently revealed that the majority of patients were ACPA negative and had low prediction scores, indicating that they are not relevant prognostic markers in UA (2010 criteria).

The very low frequency of ACPA positivity or of high prediction scores may be explained by the fact that ACPA and the majority of variables composing the prediction rule are also included in the 2010 criteria and hence have already been used for classification. Apparently, after having been used for risk estimation via the 2010 criteria, neither ACPA nor the prediction rule is able to subsequently identify the individual patients who are missed by the 2010 criteria.

It was observed that some of the 2010 UA patients already fulfil the 1987 criteria at baseline. These data confirm previous reports, showing that not all 1987 RA patients are classified as RA according to the 2010 criteria[9, 10]. This may suggest that it is appropriate to use both the 2010 and 1987 criteria in individual patients. Finally, we studied 1987 and 2010 UA patients; the findings regarding ACPA and the prediction rule in these patients were comparable to those in the 2010 UA patients.

A strength of the present study is that three different early arthritis cohorts were studied. These cohorts had slightly different inclusion and exclusion criteria, which can be seen as a limitation. Despite this heterogeneity, the observed findings were remarkably similar between cohorts, strengthening the validity of the findings and the conclusion that ACPA and the Leiden prediction rule are not accurate prognostic tools in 2010 UA patients.

There are several caveats to this study. A potential drawback is that the replication cohorts were smaller than the first cohort. On the other hand, as mentioned, the trend in the data is comparable in all three cohorts, suggesting that the results regarding ACPA and the prediction rule are not false negatives. Second, a majority of the patients studied were enrolled when aggressive DMARD treatment of UA was uncommon. Nonetheless, we cannot exclude that some of the patients who were classified as not fulfilling the 1987 criteria during follow-up are misclassified due to DMARD treatment. This implies that the

actual proportion of UA patients who have an early form of RA is even higher. However, in this scenario, the conclusions regarding the studied predictive markers would not change. Third, in a portion of the Leiden patients, ACPA data were not available, especially on those UA patients who did not progress to be 1987 criteria positive over time. Theoretically some of the patients may have been falsely classified as 2010 UA at baseline. However, considering the similarity in results between the cohorts, this influence is probably small.

In conclusion, a proportion of 1987 RA patients were not identified at baseline by the 2010 criteria. Assuming also that in UA patients who fulfil the RA criteria later in time a window of opportunity effect is present, these patients are preferably identified at baseline to this end. The ACPA test and the Leiden prediction rule are also not useful in identifying these patients. Hence other prognostic markers are needed with significantly different sensitivity and specificity characteristics to clinical examination; these may include imaging modalities such as ultrasound or extremity MRI.[14,15]

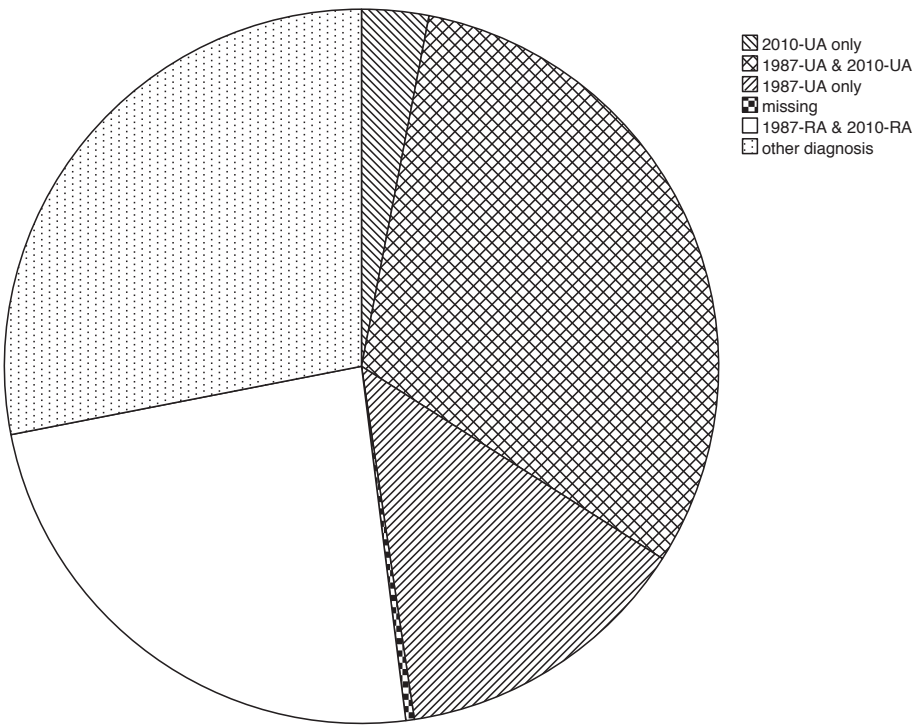
REFERENCE LIST

1. Finckh A, Liang MH, van Herckenrode CM et al. Long-term impact of early treatment on radiographic progression in rheumatoid arthritis: a meta-analysis. *Arthritis Rheum* 2006;55:864-72.
2. Furst DE, Pangan AL, Harrold LR et al. Greater likelihood of remission in rheumatoid arthritis patients treated earlier in the disease course: results from the Consortium of Rheumatology Researchers of North America registry. *Arthritis Care Res* 2011;63:856-64.
3. Quinn MA, Emery P. Window of opportunity in early rheumatoid arthritis: possibility of altering the disease process with early intervention. *Clin Exp Rheumatol* 2003; 21(5 Suppl 31):S154-7.
4. van der Linden MP, le Cessie S, Raza K et al. Long-term impact of delay in assessment of patients with early arthritis. *Arthritis Rheum* 2010;62:3537-46.
5. van der Helm-van Mil AH, le Cessie S, van Dongen H et al. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007;56: 433-40.
6. van der Helm-van Mil AH, Detert J, le Cessie S et al. Validation of a prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: moving toward individualized treatment decision-making. *Arthritis Rheum* 2008;58:2241-7.
7. Aletaha D, Neogi T, Silman AJ et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69:1580-8.
8. Britsemmer K, Ursum J, Gerritsen M et al. Validation of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: slight improvement over the 1987 ACR criteria. *Ann Rheum Dis* 2011;70:1468-70.
9. Cader MZ, Filer A, Hazlehurst J et al. Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort. *Ann Rheum Dis* 2011;70:949-55.
10. van der Linden MP, Knevel R, Huizinga TW et al. Classification of rheumatoid arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American College of Rheumatology/European League Against Rheumatism criteria. *Arthritis Rheum* 2011;63:37-42.
11. Krabben A, Huizinga TW, van der Helm-van Mil AH. Undifferentiated arthritis characteristics and outcomes when applying the 2010 and 1987 criteria for rheumatoid arthritis. *Ann Rheum Dis* 2012;71:238-41.
12. de Rooy DP, van der Linden MP, Knevel R et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology* 2011;50: 93-100.
13. van der Woude D, Young A, Jayakumar K et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009;60:2262-71.
14. Duer-Jensen A, Horslev-Petersen K, Hetland ML et al. Bone edema on magnetic resonance imaging is an independent predictor of rheumatoid arthritis development in patients with early undifferentiated arthritis. *Arthritis Rheum* 2011;63:2192-202.
15. Filer A, de Pablo P, Allen G et al. Utility of ultrasound joint counts in the prediction of rheumatoid arthritis in patients with very early synovitis. *Ann Rheum Dis* 2011;70: 500-7.

Supplementary table 1: The area under the receiver operator characteristic curve (AUC) of anti-CCP antibodies and the prediction score in '2010-UA-patients' and '1987-and-2010-UA-patients' from each of the three cohorts

	Leiden-EAC		Birmingham-EAC		Amsterdam-EAC	
	2010-UA (n=776)	1987-UA & 2010-UA (n=673)	2010-UA (n=121)	1987-UA & 2010-UA (n=114)	2010-UA (n=322)	1987-UA & 2010-UA (n=236)
Anti-CCP-positivity	0.60±0.03	0.55±0.03	0.52±0.06	0.52±0.07	0.49±0.05	0.49±0.07
Prediction rule score	0.81±0.02	0.76±0.02	0.81±0.04	0.78±0.05	0.59±0.04	0.53±0.06

Expressed are AUC +/- standard error (SE). The AUC of anti-CCP positivity was dichotomously evaluated. The AUC of the prediction rule was evaluated on the full range of scores (not categorized).



Supplementary Figure 1. In total 2,472 early arthritis patients from the Leiden EAC were studied. 772 patients were diagnosed other than unclassified arthritis or RA. Subsequently, RA was classified according to the 2010 criteria and the remaining patients were classified as 2010-UA. This resulted in 776 2010-UA patients from the Leiden EAC. 673 of these 2010-UA patients were also classified as UA according to the 1987 criteria; the '1987-UA & 2010-UA patients'. In the 4 'missing' patients only one serological test and one acute-phase response measure was obtained and therefore they could not be classified according to the 2010 criteria. The same classification was performed in the other two cohorts and resulted in 121 2010-UA patients in the Birmingham cohort and 322 2010-UA patients in the Amsterdam cohort.

Supplementary Table 2: Baseline characteristics of patients with undifferentiated arthritis by both 1987-criteria and 2010-criteria who either eventually fulfilled (RA) or did not fulfill (non-RA) the 1987-criteria during one year follow-up, in three different cohorts.

Characteristic	Leiden-EAC (n=673)		Birmingham-EAC (n=114)		Amsterdam-EAC (n=236)	
	RA (n=101)	Non-RA (n=572)	RA (n=24)	Non-RA (n=90)	RA (n=21)	Non-RA (n=215)
Morning stiffness, median (IQR) in minutes	60 (20-120)#	15 (0-60)#	90 (15-180)†	30 (0-60)†	10 (3-53)	15 (5-60)
Swollen joint count (66 joint count), median (IQR)	4 (2-6)#	2 (1-3)#	4 (2-5)†	2 (1-2)†	2 (1-4)*	2 (1-4)*
Tender joint count, median (68 joint count) (IQR)	5 (3-8)#	3 (1-5)#	4 (2-6)†	2 (1-3)†	2 (1-3)*	2 (1-5)*
ESR, median (IQR) mm/hour	24 (8-41)†	14.5 (7-32)†	18 (7.5-54)	17 (6.0-39.0)	14 (5-32)	19 (6-35)
CRP, median (IQR) mg/liter	8 (3-35.3)	7 (3-20)	14 (3-42.5)	16 (<5-47)	9 (2-23)	6 (2-21)
RF positive	11 (11)†	30 (5.3)†	2 (0.9)	1 (0.0)	2 (9.5)	12 (5.6)
ACPA positive	13 (13.4)#	17 (3.8)#	1 (0.4)	0	1 (4.8)	13 (6.0)
HAQ, mean ± SD	0.9±0.7#	0.7±0.6#	n.a.	n.a.	0.6±0.5	0.7±0.6
2010-criteria score, mean ± SD	4±1.1#	3±1.4#	4.1±0.7#	2.6±1.2#	4±0.8	4±1.1

*Leiden-EAC: 2010-UA n=776 (ACPA status n=633), 2010-UA&1987-UA n=673 (ACPA status n=539). Persistent disease: 2010-UA n=418 (ACPA status n=386), 2010-UA&1987-UA n=387 (ACPA status n=347)

**Birmingham-EAC: 2010-UA n=121 (ACPA status n=118, prediction rule n=114), 2010-UA&1987-UA n=114 (ACPA status n=111, prediction rule n=107).

***Amsterdam-EAC: 2010-UA n=322 (prediction rule n=313), 2010-UA&1987-UA n=236 (prediction rule n=227).

Except where indicated otherwise, values are the number (%) of patients. IQR = interquartile range, SD = standard deviation. *28 joint count. Characteristics were tested between RA and non-RA, per cohort. A chi-square test was used for nominal variables and the Student's t-test or Mann-Whitney U-test for continuous variables. Student's t-test was performed when variables are presented as mean and a Mann-Whitney U-test was performed when variables are presented as median. †p-value <0.05 and #p-value <0.001.

PART 2

Genetic and serologic factors in predicting radiographic progression in rheumatoid arthritis

4

Biomarkers for radiographic progression in rheumatoid arthritis

A. Krabben, T. W. J. Huizinga, A. H. M. van der Helm-van Mil

Current Pharmaceutical Design, 2014 Augustus 25. Epub ahead of print.

ABSTRACT

Treatment of patients with rheumatoid arthritis (RA) is rarely personalized, since predictors of disease course are lacking. The severity of RA can be measured objectively by radiographic progression. The most reliable way to measure radiographic progression is in a longitudinal cohort with serial time points, scoring on a quantitative scale, with a validated scoring method and trained readers.

Current models used to predict radiographic progression are based on C-reactive protein and anti-citrullinated protein antibodies. Other biomarkers could increase the prognostic ability of these models. In this review, we evaluated the published (and partly not published) data on genetic, serologic, and imaging biomarkers for the severity of joint destruction in RA.

We evaluated variants in 10 genes (*CD40*, *IL2RA*, *IL4R*, *IL15*, *OPG*, *DKK1*, *SOST*, *GRZB*, *MMP9*, and *SPAG16*). In 5 variants (*IL2RA*, *DKK1*, *GRZB*, *MMP9*, and *SPAG16*), we found evidence of an association at the functional level. We evaluated several serological biomarkers, namely, autoantibodies (RF, ACPA, anti-CarP), markers related to inflammation (ESR, CRP), and proteinases or components of the extracellular matrix of bone and cartilage (MMP3, CTX-I, CTX-II, COMP, TIMP1, PYD, RANKL/OPG, CXCL13). Finally, we evaluated markers that can be visualized by ultrasound or MRI, including erosions, bone marrow edema, synovitis, and tenosynovitis. Several studies showed that bone marrow edema and synovitis on MRI are robust predictors of radiographic progression. Some studies showed that inflammation detected with ultrasound predicted radiographic progression.

Future studies will reveal whether adding and combining all these different biomarkers will increase the accuracy of risk models predicting radiographic progression in RA.

INTRODUCTION

Providing personalized medicine in rheumatoid arthritis (RA) is one of the great challenges for the near future. In order to achieve this goal, it is necessary to fulfil 2 conditions: adequate estimation of severity so that the patients who develop severe disease can be differentiated from patients with mild disease; and identification of an individual patient's responsiveness to specific disease-modifying anti-rheumatic drugs (DMARDs) or biologics. The present review focuses on the first issue: predictors of the severity of the course of RA.

Measurement of the severity of RA

Severe RA is not uniformly defined. Patients often refer to the degree of pain or fatigue and the ability to perform daily activities and work. Rheumatologists are more concerned with the level of inflammation (expressed by the number of inflamed joints), the level of acute phase reactants, and pooled severity indices such as disease activity scores. Scientists focus on outcome measures that can be assessed objectively, such as joint destruction and mortality.[1] These perspectives are essentially similar, since levels of impaired functionality, inflammation, and structural damage are correlated.[2] Fluctuations in disease activity are directly related to changes in radiographic progression, although this relationship is less present in specific treatments such as tumor necrosis factor (TNF) blockers.[3] Joint inflammation and joint destruction both play an independent role in impaired physical functioning.[4]

Deciding which measure of severity is best depends on the reason it is selected. The advantages and disadvantages of several severity measures are summarized in Table 1. For research purposes, the rate of joint damage, as visualized on radiographs of the hands and feet, is the most common outcome measure. The main advantage is that it can be assessed objectively using a validated scoring method. The radiographs can be scored by the same reader, thus making it possible to evaluate within-reader variation and between-reader variation (in the case of ≥1 reader). Another advantage of measuring the severity of joint damage is that it accumulates over time and thus reflects disease history. In summary, the rate of joint destruction correlates with the cumulative burden of inflammation over time, is highly linked with physical function and other outcomes such as work disability, and is inexpensive to measure using validated scoring methods; consequently, the rate of joint destruction is a comprehensive endpoint in observational studies. In this review, we focus on radiographic joint damage as an outcome for identifying new risk factors for severity of RA.

Measurement of the severity of radiographic damage in RA

The absence or presence of joint damage can be assessed based on "erosiveness" or "erosive disease". This qualitative method has 2 disadvantages. First, the degree of joint damage cannot be discerned. The fact that 70%-75% of patients with early RA developed

Table 1 Measures of severity of RA and advantages and disadvantages for the use as outcome measures in studies that aim to identify biomarkers

RA severity measures	Advantages (+) and disadvantages (–)
Disease course measures	
Tender or swollen joint count	– Does not reflect cumulative severity of RA
Lansbury articular index[a]	– Fluctuates over time
DAS	
AUC of DAS over time	+ Reliable if DAS is assessed ≥3 times a year
HAQ	+ Reflects disease activity – Influenced by age, disease duration, social and psychosocial factors
Extra-articular symptoms	– Frequency nowadays decreasing thanks to introduction of potent treatment strategies
Disease outcome measures	
Mortality	+ Objective measure – May be influenced by non-RA-related causes – No increased mortality risk observed in case of DAS-guided treatment[b]
DMARD-free sustained remission[c]	+ Most favourable outcome of RA as it approximates cure – Long follow-up necessary in order to achieve this outcome and to assure that remission is sustained
Rate of radiographic joint destruction	+ Objective and well validated, quantitative scoring methods available + Quality of scoring can be easily expressed + Reflects cumulative severity of RA + No fluctuation in time – Scoring is time and cost consuming (need a trained scorer) (and eventually taken extra radiographs besides normal clinical care)

[a]In the Lansbury articular index, the joint counts are weighted for the joint size. Such weighting may be preferable, as the volume of inflamed synovial tissue would be proportional to the serum CRP level and the level of disability.
b(111;112)
[c](113)
RA, rheumatoid arthritis; DAS, disease activity scale; AUC, area under the curve; HAQ, health assessment questionnaire; DMARD, disease-modifying antirheumatic drug.

erosions within the first years of follow-up indicates that this measure is suitable in very early phases of the disease, but not in more advanced stages.[5] Second, no uniform definition for erosiveness or erosive disease exists. Performing analyses with non-validated outcome measures increases inter-observer variation and variation between studies. Indeed, the various descriptions for erosive RA include any radiographic evidence of erosions,[6,7] a cortical break of ≥2 mm (8), and presence of ≥2 or ≥3 erosions.[9;10] According to the 2010 ACR/EULAR criteria, typical erosive disease is defined as at least 3 small hand or foot joints

with an interrupted cortex. This condition was associated with a specificity of >85% for prescription of methotrexate and >90% for disease persistency.[11]

Currently used quantitative scoring methods to measure joint damage severity include the Sharp van der Heijde score (SHS) and (modified) Larsen score. These quantitative measurements are more discriminative than binary outcome measures, have been validated, and can be used in all phases of the disease.[12,13] Both the SHS and Larsen score are used to assess the joints of the hands and feet. Of the two, SHS is the only one that differentiates between bone erosions and cartilage damage, which is visualized as joint space narrowing. Compared to the Larsen score, the SHS is more sensitive for detecting changes over time, although it is also more time-consuming to apply.[12]

Given that quantitative outcomes are more accurate than qualitative outcomes and repeated outcomes are even more accurate, the number of time points is a key element of optimal assessment of joint destruction. Within-patient correlation of serial measurements is important when scoring radiographs. Unlike damage on radiographs from different patients, the severity of damage on serial radiographs from the same patient is highly correlated. Compared to measuring radiographs at only 1 time point, scoring radiographs at subsequent time points substantially diminishes within-patient variability in joint damage. Consequently, the power of the study is increased, a smaller number of patients is needed to detect a difference, and phenotypic misclassification is reduced.[14] Hence, a more precise estimation of the rate of joint destruction facilitates differentiation between true effects and noise.

Although radiographs are scored with a quantitative scoring method, sometimes only 1 radiograph per patient is available and the time point at which the radiographs were made varies between patients. Since radiographs are made at different phases of the disease, scores cannot be easily compared. In order to estimate the effect of risk factors on joint damage in this circumstance, the estimated radiographic progression per year can be determined by dividing the total radiographic score by the disease duration at the time the radiograph was taken. This approach is limited in that it assumes that the baseline score was zero and includes both patients with early RA and patients with advanced RA, thus precluding comparison of estimated radiographic progression rate. The course of joint damage is frequently linear in the early years and slows down as the disease progresses.[15] Inaccurate estimation of disease duration has a larger impact on patients with short disease duration. Consequently, the estimated radiographic progression per year may be overestimated in patients with short disease duration. An extreme situation is that of a patient with a long disease duration in which the maximal level of joint destruction has been reached but no further destruction has evolved since then; consequently, the level of joint destruction remains unchanged during further follow-up. If the single radiography is taken during this period with a stable SHS score, the estimated annual progression decreases with every year of follow-up. Therefore, the use of the estimated annual progression is

restricted to a short follow-up duration and harbors a risk of phenotypic misclassification in advanced disease stages.

In conclusion, the rate of joint destruction in longitudinal studies should be assessed at serial time points on a quantitative scale using validated scoring methods and trained readers.

Analysis of the rate of joint destruction in longitudinal studies

Radiographic data are by definition skewed, as many patients have little progression and few patients have marked progression. This skewness makes it difficult to apply statistical tests, many of which presume a normal distribution. However, a normal distribution can frequently be achieved by log-transformation of the data. Given that several radiographic measurements may be made, it is beneficial to use a statistical method that takes advantage of within-patient correlations, as this will yield more precise estimations of the progression rate and, therefore, increase the possibility of detecting differences. Repeated analyses based on covariance matrices, for instance, linear mixed models, also make it possible to include patients with missing radiographs over time. This possibility is relevant, since missing data are often not completely random but related to the severity of the disease course; over time, patients with the most severe disease can die and those with the least severe disease are often lost to follow-up. Excluding these patients will make the obtained effect size less generalizable to the general patient population. In other words, including all patients in a specific population reduces the likelihood of selection bias that will be introduced if per protocol analysis is performed.

Treatment effects and other confounding factors

The effect of potential biomarkers on the rate of joint destruction should ideally evaluated in longitudinal cohorts of patients in whom the disease course of RA is natural (ie, untreated patients). Such may be the case of patients diagnosed and recruited in the 1980s or early 1990s, although such datasets are rare today. When studying more recently diagnosed patients, analyses should be adjusted for treatment effects to discern the role of the risk marker. Other potential confounding factors are age and gender. Whereas the effect of gender is different between cohorts, the effect of age is consistent: in almost all studies, older age at onset is associated with a more severe disease course.

BIOMARKERS

A biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of a normal biological or pathogenic process; it may also be an indicator of response to a therapeutic intervention.[16]

According to this definition, the term biomarker covers any type of characteristic. Biomarkers are classified into 3 groups: genetic markers, serologic markers, and imaging markers.

An OMERACT task force proposed a much more stringent definition for a biomarker, namely, that it reflects structural damage in RA.[17] The definition is based on 14 requirements, which include not only criteria for the reliability of the assay and the discriminative ability of the marker, but also items reflecting the "truth". The criteria include the following: evidence that a biomarker reflects tissue remodeling demonstrated in animal models, immunohistochemical localization in joint tissue, and correlation between levels of the biomarker and scores of other surrogates for structural damage. These criteria were developed for serological markers but may also be applicable to other types of biomarkers. Nonetheless, this definition of a biomarker is more challenging to fulfill than the definition provided above. In this manuscript, we define biomarkers as genetic, serologic, and imaging markers that are predictive of radiographic progression in patients with RA.

Types of biomarkers for joint damage progression in RA

Twin studies have indicated that radiographic progression is in part heritable. Recent estimations on the Icelandic RA population yielded a heritability of 45%-58%.[18] The other 42%-55% can be explained by environmental or random factors. Genetic factors may predispose to serologic risk factors that are also predictive of the severity of joint damage and to joint damage via unknown mechanisms.

Serologic biomarkers are produced under the influence of both genetic and environmental factors, as illustrated by anti-citrullinated protein antibodies (ACPA). Their presence is associated with severe disease course, and certain *HLA-DRB1* alleles and smoking predispose to the development of these autoantibodies.[19]

Imaging characteristics can also act as biomarkers, as they can indicate a pathogenic process. Among the 3 categories of biomarkers discussed, serologic and imaging biomarkers are closest to the phenotype, because advanced imaging techniques actually visualize subclinical (and clinical) disease features (Figure 1).

GENETIC BIOMARKERS FOR THE SEVERITY OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

Interest in the genetic background for susceptibility to RA has grown during recent years, and more than 40 predisposing genetic variants have been identified.[20] The vast majority were identified in genome-wide studies evaluating thousands of cases and controls.

Severity of RA is studied by making comparisons within patients, thus necessitating long-term longitudinal outcome data. Such datasets are scarce and consequently; studies

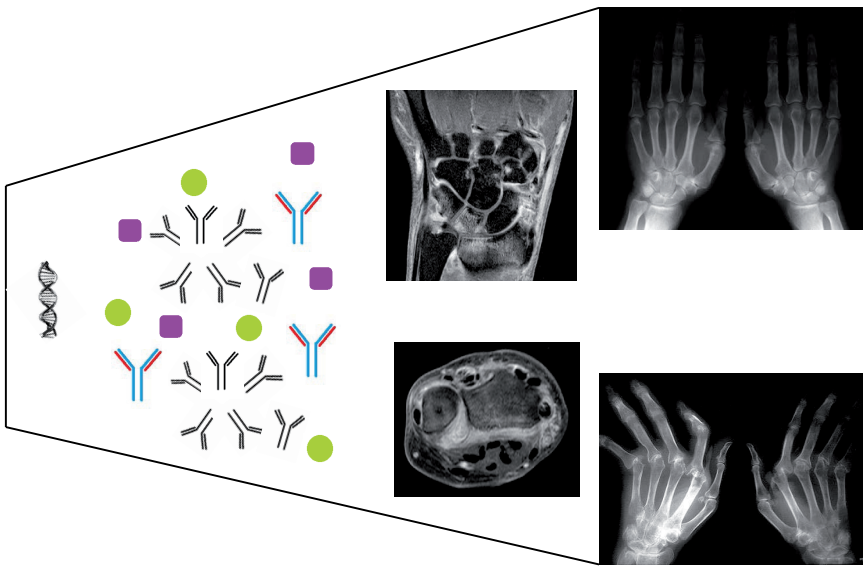


Figure 1 Illustration of different biomarkers of radiographic progression in RA; in general, the closer to the phenotype a marker is, the higher the effect size is
From left to right: genetic biomarkers (SNPs), serological biomarkers (auto-antibodies, acute-phase reactants, other markers related to inflammation or components of the bone or cartilage), imaging biomarkers (bone marrow edema, synovitis, tenosynovitis, and erosions visualized by MRI or ultrasound) and the outcome (radiographic progression or no radiographic progression). In this review, we observed that the effect increased from left to right, probably because the markers located at the right are more closely related to the phenotype.

with equal sizes as in the RA susceptibility field, are out of reach. Most genetic studies on progression of joint damage are relatively small. In addition, they were not performed with a hypothesis-free genome-wide approach, but rather investigated dedicated candidate genes.

It is difficult to know when a genetic association is real. Figure 2 depicts possible levels of evidence. The first level is the *P* value; in our view this level is insufficient to indicate whether a variant is true. In high-throughput studies, where many variants are typed and analyzed, *P* values $<5\times10^{-8}$ are generally considered to be valid. This cutoff is derived from a Bonferroni correction of 0.05 (alpha)/500,000 (number of single-nucleotide polymorphisms [SNPs] analyzed). If this number of SNPs is actually studied, the *P* value reflects a probability <5% that the finding is based on chance. However, the *P* value is also largely influenced by the number of subjects being studied. Hence, a *P* value obtained in a study including several thousands of subjects may still indicate a finding that is based on chance,

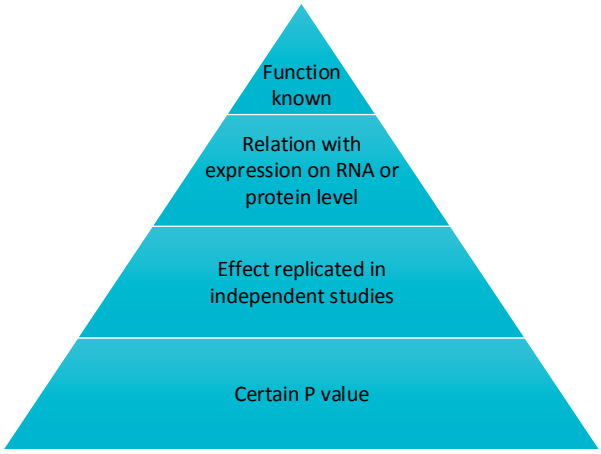


Figure 2 Level of evidence of data obtained in genetic studies; the higher up in the pyramid, the higher the level of evidence.

and a similar or higher *P* value obtained in a study containing several hundreds of patients may be indicative of a "true variant". In conclusion, using the *P* value as a measure to evaluate the reliability of findings is spurious. A more reliable method is replication. If a variant is statistically associated in several independent cohorts, the chance that the observation reflects chance finding is greatly reduced. Even more convincing are data that support the finding at a different level. Examples include genetic variants associated with changed expression at mRNA or protein level. Genetic associations that are not supported by expression data may also be genuine, although the presence of associations at the level of expression does increase reliability and may act as an initial step in better understanding the consequences of carrying a certain genetic variant. In the ideal situation, the pathway or mechanism via which a genetic risk factor influences disease is known.

During the last 2 decades, many studies have evaluated genetic markers in relation to the severity of RA. The results were often inconsistent, probably owing to small samples and different types of outcome measure.

We reviewed the literature and included unpublished data on genetic variants associated with progression of joint damage. We only reviewed genetic variants that were assessed in several populations. Variants that were assessed in only 1 cohort were not addressed, because of the low level of evidence, since the study was not replicated. The studies that were available measured joint damage in different ways, thus precluding a meta-analysis.

The candidate genes evaluated to date can be categorized as markers associated with the development of RA, markers of inflammation, and markers related to bone or cartilage.

Markers associated with the development of RA

HLA-DRB1

The *HLA-DRB1* alleles coding for the so-called shared epitope are the oldest genetic risk factor associated with progression of joint damage. This marker is also the most widely validated genetic risk factor. The presence of shared epitope alleles is a risk factor for ACPA, which are associated with progression of joint damage. Interestingly, in a recent study on progression in ACPA-negative RA, the *HLA* region was not identified as a marker for radiographic progression (manuscript under review), thus providing further evidence that the relevance of the *HLA-DRB1* region for progression lies in predisposition to development of autoantibodies. The *HLA-DRB1* region that codes for the shared epitope alleles can also code for the *DERAA* amino acid motif. The presence of this variant has been associated with protection against radiographic progression.[21] To our knowledge, this effect has never been studied in other cohorts and therefore has not been replicated. The mechanism by which *HLA-DRB1* alleles predispose to ACPA and progression of joint damage is not completely understood, although the hypothesis is that these genetic variants influence the immune response by affecting the antigen-binding site.

PTPN22

A coding variant in *PTPN22* (rs2476601) has been studied in 8 populations to establish an association with the severity of joint damage. Although the minor allele was associated with more severe progression in 2 studies,[22,23] subsequent studies in 5 other cohorts did not reveal a significant association (Table 1).[24-27] Performing a meta-analysis of these studies is difficult, owing to the different quantitative and qualitative outcome measures used.

TNFAIP3-OLIG3

Several variants in this region are associated with the risk of developing RA. rs6920220 was associated with progression of joint damage in a UK study consisting of 685 radiographs from 700 ACPA-positive RA patients.28 In a Dutch study consisting of 844 radiographs from 181 ACPA-positive RA patients, this association was not replicated,[29] and no association was present in unpublished data from several North American cohorts. (Table 2) Most available data are for rs675520, which was studied in 8 cohorts; a meta-analysis of 7 of these cohorts did not reveal a significant association with RA.[29,30]

C5-TRAF1

Although a significant association was observed for rs2900180 in the *C5-TRAF1* region in 2 UK cohorts,[31] other datasets did not reveal a significant association (Table 2). A meta-analysis of these data is required to draw conclusions on the relevance of this SNP for

progression of joint damage. An initial positive association was observed for rs10818488, which is another variant in this region; however, this association was not maintained in a meta-analysis of 7 cohorts.[30,32]

CD40

A genetic variant in *CD40* (rs4810485) was shown to be a risk factor for development of ACPA-positive RA. This marker was subsequently studied in relation to radiographic progression in 2 cohorts of ACPA-positive RA patients. A significant association was observed in both. Intriguingly, the minor allele that was associated with a decreased risk of RA was associated with more severe progression of joint damage. No ready explanation is available for this observation.[33]

IL2RA

The polymorphism rs2104286, which is found in the gene coding for the IL2 receptor α chain (*CD25*), is clearly associated with progression of joint damage. Significant associations were demonstrated in Dutch, Icelandic, and North American RA populations. Furthermore, the minor allele, which is associated with reduced progression, was also associated with lower serum levels of IL2RA, which correlated with progression.[34] A multivariate analysis including both *IL2RA* and serum IL2RA showed that only serum levels were independently associated with progression, suggesting that the genetic variant affects progression by a mechanism that also affects serum levels. rs2104286 is the only genetic variant predisposing to development and progression of RA for which functional data are available. Interestingly, this variant also predisposes to other autoimmune disorders such as multiple sclerosis and type 1 diabetes.

Variants in a further 2 susceptibility genes (*AFF3* rs11676922 and *BLK* rs13277113) have been studied in several cohorts, but no evident association with the severity of joint damage was found.

Inflammatory markers as candidates for joint damage progression

Joint damage results from deregulated inflammation or disturbances in bone or cartilage homeostasis (Figure 3). Joint destruction, as visualized on hand and foot radiographs, is the local loss of cartilage and bone resulting from inflammation. The presence of specific autoantibodies is thought to propagate the level and chronicity of inflammation and may directly and indirectly affect the level of structural damage.[35] Genes coding for inflammatory markers can also influence the level of inflammation and are therefore likely subjects for candidate gene studies focused on progression of joint damage in RA (Table 3).

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
HLA-DRB1	SE-alleles	324 RA	1301 radiographs (baseline, 1, 2, 3, and 4 years)	Modified SHS	Destructive	<0.05 (at all time points)	Van der Helm-van Mil et al.(21)
	SE*04	111 RA	At baseline and 1 and 2 years[b]	Modified Sharp: divided into 3 categories erosion gain	Destructive	0.02, 0.01, 0.05 (0, 1, and 2 years)	Goronzy et al.(114)
	SE-alleles	872 RA	827 radiographs (minimum disease duration of 3 years)	Modified LS	Destructive (only in RF–)	0.05	Mewar et al.(68)
	SE *04	87 RA	309 radiographs[c] (baseline, 1, 2, and 4 years)	LS	Destructive	0.01	Kaltenhauser et al.(66)
PTPN22	rs2476601 (+1858)	238 RA	At baseline, 1, 2, 5, and 10 years*	SHS	Destructive	0.01	Lie et al.(22)
	rs2476601 (+1858)	901 RA	901 radiographs[c] (at baseline)	Modified LS	Destructive	0.04	Marinou et al.(23)
	rs2476601 (+1858)	123 RA	At baseline, 1, 2, 4, and 6 years*	LS	Destructive	0.61, 0.06, 0.07, 0.14, 0.16 (0, 1, 2, 4, and 6 years)	Pierer et al.(25)
	rs2476601 (+1858)	302 RA	286 radiographs[c]	Erosive vs nonerosive	Destructive	0.20	Steer et al.(26)
	rs2476601 (+1858)	593 RA	1526 radiographs	SHS	Not reported	0.93	Van Nies et al.(27)
		397 ACPA+ RA	397 radiographs	SHS	Destructive	ACPA+ 0.22	
	rs2476601 (+1858)	689 RA	627 radiographs	Erosion present or absent	Destructive	0.2	Karlson et al.(24)

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
TNFAIP3-OLIG3/ 6q23	rs6920220	700 ACPA+ RA	685 radiographs (at baseline)	LS	Destructive	ACPA+ 0.02	Maxwell et al.(28)
	rs6920220	181 ACPA+ RA	844 radiographs (over 5 years)	SHS	Destructive	ACPA+ 0.76	Scherer et al.(29)
	rs6920220	600 RA (Leiden-EAC)	2846 radiographs (over 7 years)	SHS	Protective	0.53	Not published data.
		101 RA (Wichita)	337 radiographs (over 15 years)	SHS	Destructive	0.57	
		649 RA (NDB)	649 radiographs	SHS	Protective	0.25	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Destructive	0.97	
	rs675520	181 ACPA+ RA	849 radiographs (over 5 years)	SHS	Protective	ACPA+ 0.007	Scherer et al.(29)
	rs675520	2666 RA (meta-analysis of 7 cohorts)	6282 radiographs (4 repeated and 3 single measurement)	LS and SHS	Two protective and 5 destructive	0.49	Knevel et al.(115)

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
C5/TRAF1	rs2900180	445 RA	2161 radiographs (over 15 years)	LS	Destructive	0.011 (Combined data)	Viatte et al.(31)
		1446 inflammatory polyarthritis	2168 radiographs (baseline, 1 and 5 years)	LS			
	rs2900180	600 RA (Leiden-EAC)	2846 radiographs (over 7 years)	SHS	Destructive	0.056	Not published data.
		101 RA (Wichita)	337 radiographs (over 15 years)	SHS	Protective	0.988	
		649 RA (NDB)	649 radiographs	SHS	Destructive	0.371	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Protective	0.078	
	rs10818488	278 RA	556 radiographs (baseline and 2 years)	SHS	Destructive	0.008	Kurreeman et al.(32)
	rs10818488	2666 RA patients (meta-analysis of 7 cohorts)	6282 radiographs (4 repeated and 3 single measurements)	LS and SHS	Three protective and 4 destructive	0.72 (meta-analysis)	Knevel et al.(30)
CD40	rs4810485	250 ACPA+ RA	2940 radiographs (over a maximum of 9 years)	SHS	Destructive	0.003	Van der Linden et al.(33)
		393 ACPA+ RA	393 radiographs	SHS	Destructive	0.021	

Table 2. Susceptibility Markers as Genetic Markers for Severity of RA (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[a]	*P* value	Reference
IL2RA	rs2104286	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	Three protective and 1 destructive	7.2*10^-4 (meta-analysis)	Knevel et al.(34)
AFF3	rs11676922	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	Two protective and 2 destructive	0.08 (meta-analysis)	Knevel et al.(34)
BLK	rs13277113	1750 RA (meta-analysis of 4 cohorts)	4732 radiographs (2 repeated and 2 single measurements)	SHS	One protective and 3 destructive	0.11 (meta-analysis)	Knevel et al.(34)

[a]The direction of the effect size is presented, irrespective of the *P* value of the obtained result. Hence, the direction of nonsignificant findings was also presented.
[b]Number of radiographs not reported in manuscript.
[#]These data were not reported in the manuscript, but derived from the data presented in the text or tables.
[†]The minor alleles were different between the cohorts. Between brackets is the allele with the lowest frequency in the specific cohort.
ACPA, anti-citrullinated protein antibodies; AFF3, AF4/FMR2 family; BLK, B lymphocyte kinase; CD40, cluster of differentiation 40; C5/TRAF1, complement component 5/tumor necrosis factor receptor-associated factor 1; HLA-DRB1, human leukocyte antigens-DRB1; IL2RA, interleukin 2 receptor alpha, member 3; LS, Larsen score; Leiden-EAC, Leiden early arthritis clinic; NARAC, North American Rheumatoid Arthritis Consortium; NDB, National Data Bank (American cohort); SHS, Sharp van der Heijde score; PTPN22, protein tyrosine phosphatase, non-receptor type 22; RA, rheumatoid arthritis; SE-alleles, shared epitope-alleles; TNFAIP3-OLIG3, tumor necrosis factor, alpha-induced protein 3-oligodendrocyte transcription factor 3.

IL1

TNF-α and IL-1 are pro-inflammatory markers that are overexpressed in RA. However, while few studies have been performed on genetic variants in *TNFA*, several studies have addressed variants in *IL1*. The activity of IL-1 reflects the function of 2 molecules, IL-1α and IL-1ß. IL-1α is cell-bound and IL-1ß is a secreted cytokine. No associations have been reported between variants in *IL1A* and the severity of radiographic damage in RA. For *IL1B*, rs16944 was evaluated in 3 studies but no effect was observed.[23,36,37] Similarly, despite a significant association between rs1143634 in *IL1B* and both serum levels and joint destruction in a study of 297 patients and 273 radiographs,[38] further studies (2762 patients and 5956 radiographs) could not replicate this association (Table 3).[23,37,39] Based on these data, variants in *TNFA*, *IL1B*, and *IL1A* do not predispose to severe destructive RA.

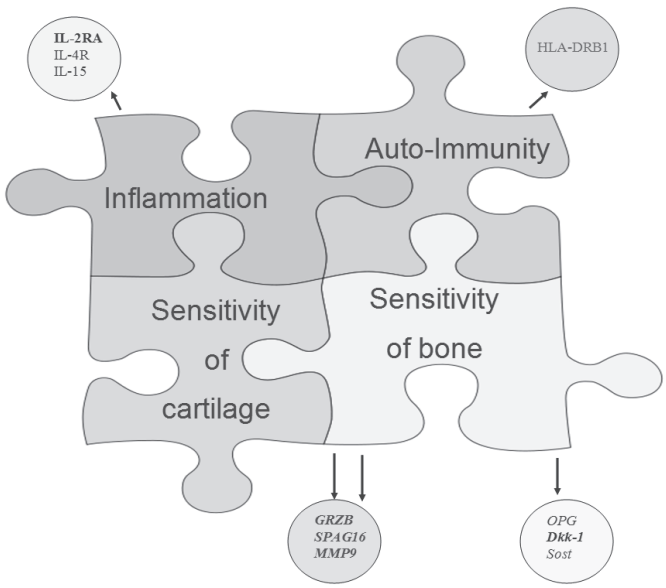


Figure 3 In RA, joint damage is the result of several processes: inflammation, auto-antibodies, and resistance of cartilage and bone against degradation.
The genetic markers that are replicated are presented; the genetic variants that are observed to be associated with differences at the level of expression are indicated in bold.
IL2RA, interleukin 2 receptor alpha; IL4R, interleukin 4 receptor; IL15, interleukin 15; HLA-DRB1, human leukocyte antigens-DRB1; GRZB, granzyme B; SPAG16, sperm associated antigen 16; MMP9, matrix metallopeptidase 9; OPG, osteoprotegerin; DKK1, dickkopf-related protein 1; SOST, sclerostin.

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity.

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL1B	rs16944 (–511)	712 RA	712 radiographs	Erosion vs no erosion	Protective	0.67	Johnsen et al.(37)
	rs16944 (–511)	932 RA	932 radiographs# (at baseline)	Modified LS	Not conclusive	0.8	Marinou et al.(23)
	rs16944 (–511)	157 RA	157 radiographs	LS plotted against disease duration	Protective	n.s.	Genevay et al.(36)
	rs1143634 (+3954)	297 RA	273 radiographs	destructive vs nondestructive acc. to wrist LS (>2 vs <2)	Destructive	0.03 (Larsen index 0.005) (HLA: 0.0001)	Buchs et al.(38)
	rs1143634 (+3954)	712 RA	712 radiographs	Erosion vs no erosion	Protective	0.54	Johnsen et al.(37)
	rs1143634 (+3954)	147 RA	147 radiographs	Right Larsen wrist score >=2 vs <2	NR	NS	Marotte et al.(39)
	rs1143634 (+3954)	880 RA	880 radiographs# (at baseline)	Modified LS	Not conclusive	0.1	Marinou et al.(23)
	rs1143634 (+3954)	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS	Protective	0.07	Not published data.
		101 RA (Wichita)	337 radiographs (during 15 years)	SHS	Protective	0.62	
		649 RA (NDB)	649 radiographs	SHS	Destructive	0.77	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Destructive	0.25	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL4	rs2243263	965 RA	892 radiographs (minimum disease duration of 3 years)	Modified LS	Not conclusive	0.3	Marinou et al.(42)
	rs2243263	600 RA	2846 radiographs (during 7 years)	SHS	NR	NS	Krabben et al. (41)
IL4R	rs1805010 (I50V)	302 RA	302 radiographs (2 years after disease onset)	Erosion vs no erosion	Destructive	<0.0001	Prots et al.(40)
	rs1805010 (I50V)	965 RA	908 radiographs (minimum disease duration of 3 years)	Modified LS	Not conclusive	0.5	Marinou et al.(42)
	rs1805010 (I50V)	600 RA	2846 radiographs (during 7 years)	SHS	NR	NS	Krabben et al. (41)
	rs1801275 (Q551R)	965 RA	912 radiographs (minimum disease duration of 3 years)	Modified LS	Protective	0.5	Marinou et al.(42)
	rs1801275 (Q551R)	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.01	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.21 (meta-analysis)	
	rs4787423	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.03	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.81 (meta-analysis)	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
	rs1805015	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.04	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	NR	0.08 (meta-analysis)	
	rs7191188	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	<0.01	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	NR	0.83 (meta-analysis)	
	rs6498016	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	0.01	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	NR	0.88 (meta-analysis)	
	rs1805011	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.01	Krabben et al. (41)
		1203 RA (meta-analysis of 4 cohorts)	2429 radiographs (2 repeated and 2 single measurement)	SHS and LS	Four destructive	0.02 (meta-analysis)	
	rs1119132	600 RA	2846 radiographs (during 7 years)	SHS	Destructive	0.04	Krabben et al. (41)
		1953 RA (meta-analysis of 6 cohorts)	3415 radiographs (3 repeated and 3 single measurement)	SHS and LS	Three destructive and three protective	0.001 (meta-analysis)	

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL6	rs1800795 (–174)	930 RA	930 radiographs# (at baseline)	Modified LS	Protective	0.005	Marinou et al.(23)
	rs1800795 (–174)	600 RA	2846 radiographs (during 7 years)	SHS	Protective	0.64	Not published data.
	rs1524106	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and two destructive	0.006 (meta-analysis)	Not published data.
	rs7796691	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and three destructive	<0.001 (meta-analysis)	Not published data.
	rs7776857	1418 RA (meta-analysis 4 cohorts: Leiden-EAC, Groningen, Lund, Sheffield)	4890 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and two destructive	0.191 (meta-analysis)	Not published data.

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL10	rs1800896 (–1082)	108 early RA	At 2 years of follow-up*	Erosion vs no erosion	NR	NS	Cantagrel et al.(44)
	rs1800896 (–1082)	91 RA	At baseline, 3, 6 and 12 years*	SHS	Destructive (G) [†]	<0.02	Huizinga et al.(43)
	rs1800896 (–1082)	860 RA	860 radiographs# (at baseline)	Modified LS	Protective (A) [†]	0.01	Marinou et al.(23)
	rs1800896 (–1082)	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS	Protective (A) [†]	0.92	Not published data.
		101 RA (Wichita)	337 radiographs (during 15 years)	SHS	Protective	0.122	
		649 RA (NDB)	649 radiographs	SHS	Protective	0.84	
		385 ACPA+ RA (NARAC)	385 radiographs	SHS	Protective	0.09	
	rs1800872 (–592)	928 RA	928 radiographs# (at baseline)	Modified LS	Protective	0.006	Marinou et al.(23)
	Haplotype: –1082, –819, –592	95 RA	At baseline*	Erosive vs. non erosive	NA	NS	Pawlik et al.(45)

Table 3 Candidate Gene Studies Evaluating Genes Encoding for Inflammatory Markers as Genetic Markers for RA Severity. (continued)

Gene	SNP	Patients	Radiographs	Outcome	Effect of minor allele protective/ destructive[∞]	*P* Value	Reference
IL15	rs2322182	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	NS (meta-analysis)	Knevel et al.(46)
	rs7667746	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	4.8*10^-5 (meta-analysis)	Knevel et al.(46)
	rs7665842	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	3.6*10^-4 (meta-analysis)	Knevel et al.(46)
	rs4371699	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	0.01 (meta-analysis)	Knevel et al.(46)
	rs6821171	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and one destructive	0.01 (meta-analysis)	Knevel et al.(46)
TGFb	–869	173 RA	173 radiographs (at baseline)	LS	Protective	NS	Mattey et al.(51)
	–869	122 RA	122 radiographs	Modified Sharp score	Destructive	NS	Kim et al.(50)
FCRL3	rs7528684	117 RA	234 radiographs (at baseline and 10 years)	SHS progression	Destructive	0.02	Maehlen et al.(48)
	rs7528684	227 RA	227 radiographs (mean disease duration 6.3)	Modified Sharp score	Destructive	0.003	Han et al.(47)
	rs7528684	600 RA (Leiden-EAC)	2846 radiographs (during 7 years)	SHS plotted against disease duration	Destructive	0.57	Not published data.

w[∞]The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
[#]These data were not reported in the manuscript, but derived from the data presented in the text or tables.
[†]The minor alleles were different between the cohorts. The allele with the lowest frequency in the specific cohort appears in parentheses.
RA, rheumatoid arthritis; ACPA, anti-citrullinated protein antibodies; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant; IL1B, interleukin 1 beta; IL4, interleukin 4; IL4R, interleukin 4 receptor; IL6, interleukin 6; IL10, interleukin 10; IL15, interleukin 15; TGFb, transforming growth factor beta; FCRL3, FC receptor-like protein 3; Leiden-EAC, Leiden early arthritis clinic; NARAC, North American Rheumatoid Arthritis Consortium; NDB, National Data Bank (American cohort).

IL4 and IL4R

SNPs in *IL4* and *IL4R* have been studied in several RA populations. IL4 is considered relevant, as it mainly promotes differentiation of T cells towards T_H2 cells. The role of IL4 is underscored by observations that IL4 knockout mice are characterized by extensive joint damage and that low synovial concentrations of IL4 have been detected in patients with established RA. Nevertheless, no clear associations have been detected between genetic variants in *IL4* and severity or progression of joint damage. The effect of IL4 is mainly mediated via the IL4Rα chain. An amino acid–changing variant in *IL4R* (rs1805010) was shown to associate with the presence of erosions.[40] Some indications were also found for functional readouts in this variant. Nonetheless, subsequent studies in UK and Dutch RA populations could not replicate the association between rs1805010 and progression of joint damage.[41,42] A recent study of tagging SNPs in *IL4R* observed and independently replicated 2 additional *IL4R* variants associated with radiographic progression (rs1119132 and rs1805011).[41] These 2 variants were in low linkage disequilibrium ($R^2 < 0.01$) with rs1805010. No data are available on the functional level of these 2 variants.

IL6

Marinou et al. found that rs1800795 in *IL6* was associated with less severe joint damage.[23] This association was not replicated in a Dutch cohort (Table 3), in which 3 other variants were associated with the severity of joint damage and were subsequently studied in other cohorts; 2 of these variants were significantly associated with joint damage in a meta-analysis that included the Dutch cohort (Table 3). Without the Dutch cohort, the association was no longer significant. Based on this observation and the difference in effect direction between the cohorts, no clear conclusions can be drawn on genetic variants in *IL6* and progression of joint damage.

IL10

Several lines of research suggest an important role for IL10 in the pathogenesis of joint destruction in RA. Preclinical studies showed that IL10 inhibits the generation of proinflammatory cytokines and the proliferation of T_H1 cells. In a rodent model of chronic arthritis, IL10 inhibited the severity of the disease.[43] The minor allele of a promoter polymorphism at position –1082 (rs1800896) was associated with less severe joint damage in a cohort of 91 female RA patients.[43] A similar significant observation was made in a UK study.[23] Furthermore, not significant findings were observed with a similar effect direction in several cohorts (Table 3).[44] Therefore, it would be very interesting to perform a meta-analysis of this variant, especially given the correlation observed between rs1800896 and expression, since patients with the severity risk allele produced lower IL10 levels. Similar findings were obtained by studying haplotypes of the –1082A/G, –819C/T, and –592A/C variants.[45] The –592 (rs1800872) variant in this haplotype ($R^2 = 0.29$ with rs1800896) was

also found to be associated with the severity of joint damage in one study, although other studies did not support this finding.[23;45] Whole-genome association studies showed that variants in the *IL10* gene were a relevant risk factor for Crohn disease and Behçet disease.

IL15

IL15 levels are increased in the serum, synovium, and bone marrow of patients with RA. IL15 influences both the innate and the adaptive immune response; it is mainly responsible for activation and proliferation of T cells. Emerging data show that this cytokine affects osteoclastogenesis. A tagging approach revealed several variants in *IL15* to be associated with the severity of progression of joint damage. Furthermore, in a meta-analysis of 4 cohorts, 4 variants were associated with progression.[46]

FCRL3

The Fc receptor–like family has potential immunoregulatory functions. FCRL3 has attracted research interest, since it is associated with several autoimmune diseases. It has been observed to be a risk factor for development of RA in Asian patients. It is preferentially expressed in B cells, and the –169C allele of *FCRL3* (rs7528684) has been associated with higher ACPA levels. In a Korean study, RA patients with the CC genotype have higher radiographic progression rates.[47] In a Norwegian study, ACPA-positive patients carrying both CC alleles also had more severe progression.[48] In a large Dutch cohort, no association was observed between *FCRL3* genotypes and radiographic progression in RA or ACPA-positive RA (Table 3). A meta-analysis of these data would be required to determine whether or not *FCLR3* is associated with severity of RA.

TGFβ1

The *TGFβ1* –869T/C variant was observed to be a susceptibility factor for RA in the Japanese population.[49] Subsequently, the *TGFβ1* –869T/C variant, which is related to the severity of joint damage, was investigated in 2 studies. In a Korean study, no significant association was observed.[50] A study from the UK found an association between this variant and joint damage, although the association was no longer significant when disease duration at the point the radiographs were taken was included in the analysis.[51] Considering the available data, this variant is not evidently associated with severity of joint damage.

Markers related to bone or cartilage

Destruction of bone or cartilage and the ability of bone and cartilage to resist inflammatory pressure may in part be explained by the patient's genetic constitution. Several markers have been studied to this end (Table 4).

RANK, RANKL, OPG, and TRAF6

The balance between osteoblast and osteoclast activity is crucial for healthy bone, and osteoclast-related bone loss is mediated by the OPG/RANK/RANKL/TRAF6 pathway. Receptor activator for nuclear factor κβ ligand (RANKL) is expressed and released by osteoblasts and activated T lymphocytes. RANKL promotes osteoclast formation and perpetuates its function and survival through binding of receptor activator of nuclear factor κβ (RANK). Subsequently, the RANK signal is mediated by TRAF6, a member of the TNF receptor associated factor (TRAF) protein family, which functions as a signal transducer in the NF-κβ family. The process of osteoclast formation and bone resorption is negatively regulated by osteoprotegerin (OPG), as binding of OPG inhibits activation of RANKL. The bone loss in RA points to an imbalance in the OPG-RANKL axis favoring bone resorption and resulting in erosion. This potential imbalance is supported by the association between the OPG/RANKL ratio in serum and joint destruction in RA. Genetic variants in *OPG*, *RANK*, and *RANKL* have been associated with bone mineral density and osteoporosis, and *TRAF6* has also been identified as a risk factor for development of RA. The variants tagging these 4 genes were evaluated recently in a candidate gene study. Variants that were significantly associated in the first cohort were subsequently studied in 3 additional cohorts. None of the variants in *RANK*, *RANKL*, or *TRAF6* were replicated after correction for multiple testing, although 1 variant in *OPG*, rs1485305, was significantly associated with more severe joint damage in a meta-analysis of the 4 cohorts and again after Bonferroni correction. This variant has also been associated with loss of bone mineral density (unpublished data) (Table 4).

DKK1, SOST, LRP5, and KREMEN1

Another pathway that is relevant for bone homeostasis is the canonical WNT/ß-catenin pathway, which involves binding of WNT proteins to a co-receptor complex comprising LRP5 or LRP6 and a member of the frizzled family of proteins. This binding leads to a signalling cascade resulting in the release of catenin in the cytoplasm, which eventually stimulates osteoblast differentiation. The cascade is negatively regulated by dickkopf 1 (DKK1) and sclerostin (SOST). DKK1 can also bind to cell surface receptor KREMEN1 and LRP5, thus strengthening the negative regulatory effect. Genetic variants in *LRP5* and *KREMEN1* were explored but not associated with the severity of progression of joint damage. However, several variants in *DKK1* and *SOST* were associated with progression of structural damage. In particular, when the severity risk alleles of both variants were present, a gene-gene interaction was observed, and patients with 4 risk alleles had very severe progression of damage. The relevance of the *DKK1* variants was substantiated by the finding that the risk genotypes were associated with higher serum DKK1 levels and that higher serum levels were associated with more severe joint damage in other studies. These data support the relevance of DKK1 for progression of joint damage (Table 4).[52]

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
RANK	rs17719830	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.04 (meta-analysis)	Knevel et al. *Submitted*
	rs17069845	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.09 (meta-analysis)	Knevel et al. *Submitted*
	rs11665260	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.10 (meta-analysis)	Knevel et al. *Submitted*
	rs12970081	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.08 (meta-analysis)	Knevel et al. *Submitted*
	rs8092336	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.003 (meta-analysis)	Knevel et al. *Submitted*
	rs6567279	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.02 (meta-analysis)	Knevel et al. *Submitted*
	rs17666267	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.01 (meta-analysis)	Knevel et al. *Submitted*
	rs8097062	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.22 (meta-analysis)	Knevel et al. *Submitted*
RANKL	rs931273	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.05 (meta-analysis)	Knevel et al. *Submitted*

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
OPG	rs1353171	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.04 (meta-analysis)	Knevel et al. *Submitted*
	rs2326045	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.15 (meta-analysis)	Knevel et al. *Submitted*
	rs10955911	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.27 (meta-analysis)	Knevel et al. *Submitted*
	rs3102724	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	Knevel et al. *Submitted*
	rs2073618	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.001 (meta-analysis)	Knevel et al. *Submitted*
	rs1564861	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.01 (meta-analysis)	Knevel et al. *Submitted*
	rs1825511	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	Knevel et al. *Submitted*
	rs1485305	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four destructive	0.0002 (meta-analysis)	Knevel et al. *Submitted*
	rs1905785	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.41 (meta-analysis)	Knevel et al. *Submitted*
	rs1905776	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.17 (meta-analysis)	Knevel et al. *Submitted*
	rs6993813	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.59 (meta-analysis)	Knevel et al. *Submitted*

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
DKK1	rs1896368	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	0.001 (meta-analysis)	De Rooy et al.(52)
	rs10762715	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
	rs1528873	1022 RA (meta-analysis of 3 cohorts)	4494 radiographs (3 repeated)	SHS and LS	Three destructive	0.009 (meta-analysis)	De Rooy et al.(52)
	rs1441124	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
	rs1896367	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Four protective	0.003 (meta-analysis)	De Rooy et al.(52)
	rs11001702	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.84 (meta-analysis)	De Rooy et al.(52)
	rs1194750	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.03 (meta-analysis)	De Rooy et al.(52)
SOST	rs4792909	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and 1 destructive	0.01 (meta-analysis)	De Rooy et al.(52)
	rs6503475	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Two protective and 2 destructive	0.03 (meta-analysis)	De Rooy et al.(52)
	rs12600549	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.07 (meta-analysis)	De Rooy et al.(52)
KREMEN1	rs1322774	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.05 (meta-analysis)	De Rooy et al.(52)
LRP5	rs3736228	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	NR	0.06 (meta-analysis)	De Rooy et al.(52)

Table 4 Candidate Gene Studies Evaluating Genes Encoding for Bone and Cartilage Markers as Genetic Markers for RA Severity (continued)

gene	SNP	Patients	Radiographs	Outcome	Effect size minor allele protective/ destructive#	P Value	Reference
GRZB	rs8192916	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	7.8*10^-4	Knevel et al.(53)
ADAMTS5	rs9984329	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	One protective and 3 destructive	0.46	Not published data.
	rs233601	1418 RA (meta-analysis of 4 cohorts)	4885 radiographs (3 repeated and 1 single measurement)	SHS and LS	Three protective and 1 destructive	0.75	Not published data.
MMP3	5A/6A	96 early RA	192 radiographs (at baseline and 4 years)	SHS	Protective (5A)[∞]	0.04	Constantin et al.(57)
	5A/6A	308 RA	839 radiographs# from three time points	Ratingen score (modification of LS)	Destructive	NS	Dörr et al.(59)
	5A/6A	254 RA	254 radiographs	LS	Destructive (6A) [∞]	0.04	Mattey et al.(58)
MMP9	rs11908352	600 RA (Leiden-EAC)	3143 radiographs (during 7 years)	SHS	Destructive	1.75*10^-7	De Rooy et al. (60)
	rs11908352	686 RA (Wichita and NDB	926 radiographs (1 repeated and 1 single measurements)	SHS	Destructive	0.002	De Rooy et al. (60)

[∞]The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
#These data were not reported in the manuscript, but derived from the data presented in the text or tables.
†The minor alleles were different between the cohorts. The allele with the lowest frequency in the specific cohort appears in parentheses.
RA, rheumatoid arthritis; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator for nuclear factor κB ligand; OPG, osteoprotegerin; DKK1, dickkopf-related protein 1; SOST, sclerostin; KREMEN1, kringle containing transmembrane protein 1; LRP5, low density lipoprotein receptor-related protein 5; GRZB, granzyme B; ADAMTS5, a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 5; MMP3, matrix metallopeptidase 3; MMP9, matrix metallopeptidase 9.

GRZB

Granzyme B (GRZB) is a serine protease found in lytic granules of NK cells and cytotoxic T lymphocytes.[53] In vitro studies showed that granzyme B has enzymatic activity for the cleavage of aggrecan proteoglycans from cultured cartilage matrix.[53] The observations that loss of cartilage proteoglycans is an early event in the course of destructive arthritis and that many granzyme B–positive cells are present in the pannus of patients with RA increased interest in GRZB as a biomarker for progression of joint damage. Four cohorts were studied, and 1 polymorphism (rs8192916) was shown to increase the risk of a more destructive course of RA. Furthermore, mapping expression of quantitative trait loci in whole blood revealed that the risk alleles were also associated with higher levels of mRNA expression.[53]

ADAMTS5

ADAMTS5, previously known as aggrecanase 2, is a member of the large ADAMTS family of zinc-dependent proteases. Aggrecan is a major proteoglycan that is responsible for the compressibility and stiffness of cartilage.[54] One of the earliest changes observed in arthritis is depletion of cartilage aggrecan due to increased proteolytic cleavage within the interglobular domain.[55] Two major cleavage sites have been identified, and ADAMTS5 is thought to cleave aggrecan at one of these sites. Murine osteoarthritis models and inflammatory arthritis models supported the relevance of ADAMTS5 for aggrecan degradation.[56] Based on the hypothesis that variants in *ADAMTS5* might influence the severity of progression of joint damage, a candidate gene study was performed in 4 cohorts that were scored according to the Sharp van der Heijde method (in which the joint space narrowing score reflects the severity of loss of cartilage). No clear associations were observed in the 1418 patients and 4885 radiographs studied (Table 4).

MMP3

Matrix metalloproteinases (MMP) comprise a group of zinc- and calcium-dependent enzymes that are implicated in the destruction of articular cartilage and bone. MMP3 is abundantly present in the synovium and synovial fluid of RA patients and is considered to be the main MMP in cartilage degradation. It is secreted by fibroblasts, synovial cells, and chondrocytes and activates other MMPs, such as MMP9 and MMP2. Serum levels of MMP3 are elevated in both early and advanced RA, and elevated serum levels are correlated with more severe joint damage. Two studies reported a significant association between the promoter polymorphism *5A/6A* and joint destruction in patients with RA.[57,58] A third study did not find this association for *MMP3 5A/6A* itself, but identified a haplotype in this region that predisposed to more severe joint destruction.[59] Other authors reported an association between the *5A/6A* polymorphism and MMP3 serum levels and found that this promoter polymorphism was functionally relevant. Altogether, these data indicate an association

between this variant and the severity of joint damage in RA. Unfortunately, this *5A/6A* variant is not included in regular high-throughput platforms, and no data are available on the association between this variant and severity of joint damage in larger cohorts.

MMP9

Fewer data are available on the association between MMP9 and progression of RA. However, a genetic variant in *MMP9* has recently been identified as a risk factor for progression of joint damage (rs11908352). The variant was not identified using a candidate gene approach, but by evaluating polymorphisms located on 186 loci that were associated with autoimmune disorders and included on the Immunochip. rs11908352 was inserted into this platform because it is located near *CD40*. Nevertheless, it was observed to be associated with progression of joint damage, independently of variants in *CD40*. Furthermore, the risk allele for severity was also associated with higher serum MMP9 levels at disease onset. These data support the relevance of MMP9 for progression of joint damage in RA.[60]

Genome-wide study of progression of joint damage

In addition to the candidate gene studies described above, one genome-wide study investigated the ACPA-positive subset of RA. The study was performed in 3 stages. In the first stage, a cluster of SNPs located at chromosome 2q34 was found to be associated with progression of joint damage. In the second and third stages, rs7607479 was replicated as a risk factor for progression. The effect was protective, as patients carrying the minor allele had less severe joint damage. This polymorphism is located within the gene coding for sperm-associated antigen 16 (*SPAG16*), which is expressed in the joint and, more specifically, in fibroblast-like synoviocytes. Fibroblast-like synoviocytes carrying the minor allele expressed and secreted less MMP3, and RA patients with the minor allele had lower MMP3 levels in their serum. Subsequently, serum levels of MMP3 were associated with the severity of joint damage. These data point to a novel factor mediating MMP3 production and progression of joint damage and were further supported by the results of a multivariate analysis showing that when both the genetic variant and serum levels were included in the model, only serum levels were independently associated with progression of joint damage. Consequently, the effect of rs7607479 on joint damage is mediated via an effect on MMP3 production.[61]

Genetic factors: Conclusion

Many studies have evaluated genetic factors as risk factors or biomarkers for progression of joint destruction in RA. Given that genetic markers generally have small effect sizes in complex disorders, the number of patients and radiographs included in many of these studies was relatively low; consequently, the power of several studies was also low. Therefore, meta-analyses combining data from all available cohorts are necessary in order

to draw robust conclusions. For several of the markers discussed here, such meta-analyses are difficult with published data, since the outcome measures used are different. The genetic variants for which it would be particularly interesting to combine the data available in meta-analyses are rs2900180 in *C5/TRAF1*, rs1800896 in *IL10*, rs1800795 in *IL6*, and rs758684 in *FCLR3*. Moreover, current data suggest that the *5A/6A* variant in *MMP3* is also associated with joint damage. Further validation in larger studies would be useful.

Nonetheless, meta-analyses have been performed and published, and in all of the cohorts included, the severity of progression of joint damage was measured quantitatively (Sharp van der Heijde and Larsen scores). Variants in 10 genes have been significantly associated with progression of joint damage in several independent studies and in meta-analyses (Figure 2). These variants are related to inflammation (eg, *IL2RA*, *IL4R*, and *IL15*), autoimmunity (eg, *HLA-DRB1*), bone homeostasis (eg, *DKK1*, *SOST*, *OPG*), and cartilage destruction (eg, *GRZB*). Some of these genetic variants were also associated with different expression levels in blood serum and blood plasma (eg, *IL2RA, DKK1, MMP9, GRZB*).

SEROLOGIC BIOMARKERS OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

Serologic markers associated with disease severity include autoantibodies, other markers related to inflammation and proteinases, and components of the extracellular matrix of bone and cartilage (Table 5).

Autoantibodies

Many studies have shown that both rheumatoid factor and ACPA are independently associated with a destructive disease course.[62-70] The most widely studied ACPA are the anti-CCP2 antibodies. Van der Linden et al compared the anti-CCP2 test with the anti-CCP3 test and the anti-MCV test for predicting radiographic progression in early RA patients. When each test was performed alone, all 3 had comparable predictive abilities. Furthermore, when the results of the anti-CCP2 test were known, the other tests had no additive value, indicating that a single ACPA test is sufficient for estimation of risk in RA.[71]

Other autoantibodies have also been explored in RA. One such autoantibody acts directly against the protein arginine deaminase type 4 (PAD4). PAD are enzymes that catalyze the citrullination reaction. PAD4 has been detected in inflamed synovium, and anti-PAD4 antibodies were associated with joint damage and erosions in a single cross-sectional study.[72] However, no confirmative studies have been reported since. Novel autoantibodies also include the anti-Carp antibodies (anti-carbamylated protein antibodies). Carbamylation is also a post-translational modification. This reaction is enhanced in smokers, in patients with renal failure, and in (chronic) inflammation. Anti-Carp antibodies are prevalent in RA. In ACPA-negative patients they were associated with the severity of joint destruction in

Table 5 Serological Markers of Bone and Cartilage Damage.

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
MMP3	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(76)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	<0.0001	Young-Min et al.(79)
	36 RA	72 radiographs (at baseline and 2 years)	Progression in SHS	NR	NS	Den Broeder et al.(80)
	109 RA	At baseline, 1 and 2 years*	Progression in SHS	Destructive	0.001	Tchetverikov et al.(75)
	98 early RA	196 radiographs# (at baseline and 1 year)	Progression in LS	Destructive	<0.05	Green et al.(116)
	26 early RA	78 radiographs (at baseline, 6 months, 12 months)	Progression in LS	Destructive	<0.05	Yamanaka et al.(117)
	32 RA	At baseline, after the first 6 months (with radiographic progression) and after the second 6 months (with no radiographic progression)*	SHS radiographic progression (≥ 5 points) vs no radiographic progression (≤ 1 point)	Destructive	<0.05	Posthumus et al.(118)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Destructive	<0.01	Roux-Lombard et al.(81)
	46 RA	At baseline and every 6 months to 2 years*	LS: erosive vs non-erosive	Destructive	0.02	Jensen et al.(83)
CTX-I (b-C-telopeptide)	279 early oligo- and polyarthritis	At baseline and after 2 years*	Progression ≥5 SHS	Destructive	<0.001	Jansen et al.(77)
	190 RA	380 radiographs (at baseline and 2 years)	Erosion vs no erosion	Destructive	0.15	Le Loët et al.(78)
	110 RA	At baseline, week 28, week 56 and at end of study (median of 4 years)*	Modified SHS: annual progression rate	Destructive	0.01	Garnero et al.(82)

Table 5 Serological Markers of Bone and Cartilage Damage. (continued)

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
CTX-II	110 RA	at baseline, week 28, week 56 and at end of study (median of 4 years)*	Modified SHS: annual progression rate	Destructive	0.02	Garnero et al.(82)
	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(82)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.003	Young-Min et al.(79)
COMP	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.004	Young-Min et al.(79)
	36 RA	72 radiographs (at baseline, 1 and 2 years)	Progressor vs nonprogressor	Destructive	0.01	Den Broeder et al.(80)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Not conclusive	NS	Roux-Lombard et al.(81)
	183 early RA	283 radiographs (at 5 and 10 years)	LS	Destructive	<0.05 (5 years) >0.05 (10 years)	Lindqvist et al.(67)
TIMP1	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.02	Young-Min et al.(79)
	24 RA	At baseline and yearly during 5 years*	LS: 3 groups: slow, intermediated and rapid progression	Not conclusive	NS	Roux-Lombard et al.(81)

Table 5 Serological Markers of Bone and Cartilage Damage. (continued)

Marker	Patients	Radiographs	Outcome	Effect elevated marker level at baseline	P Value	Reference
PYD	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.04	Young-Min et al.(79)
	190 RA	380 radiographs (at baseline and 2 years)	Erosion vs no erosion	Destructive	0.0004	Le Loët et al.(78)
	46 RA	At baseline and every 6 months to 2 years*	LS: erosive vs non-erosive	Destructive	0.07	Jensen et al.(83)
	437 RA	At baseline and yearly during 7 years*	SHS	Destructive	0.0001	Krabben et al. *in press*(84)
Glc-Gal-PYD	116 early RA	232 radiographs# (at baseline and 1 year)	Progression in modified Sharp score	Destructive	<0.01	Garnero et al.(76)
	118 RA	236 radiographs (at baseline and 2 years)	Progression in LS	Destructive	0.04	Young-Min et al.(79)
OPG / RANKL ratio	92 RA	at baseline and yearly during 5 years*	Modified SHS	Protective (OPG: RANKL)	0.001	Geusens et al.(86)
	44 RA	60 radiographs#	LS	destructive (OPG) protective (RANKL)	0.01 (OPG) 0.85 (RANKL)	Skoumal et al.(85)
	100 early RA	Mean of eight radiographs per patient (max follow-up of 11 years)*	SHS: annual progression rate	Destructive (RANKL:OPG)	<0.001	Van Tuyl et al.(87)
CXCL13	74 RA (BeSt)	At 4 years diease duration	SHS	Destructive	0.02	Meeuwisse et al.(88)
	155 RA (EAC)	At baseline and yearly during 7 years*	SHS	Destructive	<0.001	Meeuwisse et al.(88)

°°The direction of the effect size is presented, irrespective of the p-value of the obtained result. Hence, the direction was presented also of non-significant findings.
*Number of radiographs not reported in manuscript.
#These data were not reported in the manuscript, but derived from the data presented in the text or tables.
RA, rheumatoid arthritis; SHS, Sharp van der Heijde score; LS, Larsen score; NR, not reported; NS, not significant.
MMP3, matrix metallopeptidase 3; CTX-I, carboxy-terminal collagen crosslink I; CTX-II, carboxy-terminal collagen crosslink II; COMP, cartilage oligomeric matrix protein; TIMP1, tissue inhibitor of metalloproteinases 1; PYD, pyridinoline; Glc-Gal-PYD, glycosylated pyridinoline; OPG/ RANKL ratio; osteoprotegerin/ receptor activator of NF kB ligand ratio; CXCL13, chemokine (C-X-C motif) ligand 13;
BeSt, 'behandel strategieën voor rheumatoïde arthritis'; EAC, early arthritis clinic.

an early RA population.[73] Since this association has thus far not been replicated, the value of these novel antibodies as biomarkers for progression of joint damage in RA remains undetermined.

Acute-phase reactants

Since RA is an inflammatory disease, it is no surprise that C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) are associated with the severity of disease course. Nonetheless, these markers explain only a fraction of the total variance in joint destruction. A recent study calculated the variance of joint destruction explained by cumulative inflammation (area under the curve of serial CRP levels over time) to be 15-19%.[74] Hence, other markers or processes also play a role.

MMP3

The function of MMP3 has been discussed above. There is overwhelming evidence that serum MMP3 levels are associated with progression of joint damage, as a positive association has been reported in 8 of the 9 studies on this subject. The only study that did not reveal an association was small and analyzed only 36 patients. Intriguingly, several of the other studies that reported higher serum levels to be associated with future joint damage were small (24-46 patients). Given that hundreds of patients were necessary to identify genetic variants, it is clear that the effect size of serum markers is generally larger than that of genetic markers. It is noteworthy that MMP3 levels are increased throughout disease course, thus making it a stable biomarker of progression.[75]

CTX-I and CTX-II

Urinary C-terminal crosslinking telopeptide type I (CTX-I) and type II (CTX-II) collagen are markers of bone and cartilage degradation. CTX-I was associated with the severity of joint damage in all the studies that measured this parameter.[76,77] Only one study could not detect this association, although it evaluated the presence but not the severity of erosive disease.[78] Interestingly, CTX-I was a potent predictor whose effect was independent of the association between progression of joint damage and rheumatoid factor, disease activity score, or ESR. CTX-II is a specific marker of type II collagen cleavage in cartilage. Excretion of CTX-II was predictive of future joint damage, independently of other inflammatory markers.[76,79] None of these studies made adjustments for ACPA status.

COMP

Cartilage oligomeric matrix protein (COMP) is expressed at high levels in the matrix of chondrocytes. This marker was increased in patients with a more destructive disease course. It is interesting to note that significant associations were established in relatively small studies. It has not been determined whether or not the association between COMP

and joint damage was independent of other biomarkers.[67,79-81] In the largest study to date (containing 183 RA-patients), serum COMP was an independent predictor for joint damage after 5 years, although after 10 years of disease, this association was lost and only anti-CCP and CRP were independently associated with the severity of damage in hand and foot joints.[67]

TIMP

It is unclear whether tissue inhibitor of metalloproteinases 1 (TIMP) is a biomarker for severe destructive RA. Two studies have been published, both with the quantitative Larsen score as the outcome; an association was observed in one study but not in the other.[79,81]

PYD

Pyridinoline (PYD) is a major cross-linking compound of collagen fibers in cartilage that is present in the collagen of bone and tissues such as synovium. Pyridinoline levels are higher in RA patients than in healthy persons and patients with other rheumatologic disorders. In addition, some cross-sectional studies indicated that pyridinoline levels are higher in cases of active or severe RA. Prospective studies have been performed based on serum and urine pyridinoline levels. Both markers were elevated in patients who developed more severe joint destruction.[78,79,82-84] Intriguingly, this serum marker was also predictive in the early and advanced stages of RA, suggesting that it is also a stable biomarker for severity of joint damage in RA.[84]

RANKL/OPG

The genetic variants in OPG and RANKL have been discussed above. OPG is a soluble decoy receptor produced by osteoblasts that inhibits differentiation of the osteoclast precursor by neutralizing the receptor activator of NF-κβ ligand (RANKL). Although it was first observed that serum OPG levels were associated with joint damage in RA,[85] a subsequent study by Geussens et al revealed that the RANKL/OPG ratio in particular is predictive of joint destruction.[86,87]

CXCL13

CXCL13 is also known as B lymphocyte chemo-attractant and has been reported to interact with the receptor CXCR5, which is expressed by B cells and follicular B helper T cells. High levels of CXCR5 were also found in human osteoblasts, and activation by its ligand CXCL13 induced the release of extracellular matrix–degrading enzymes. CXCL13 levels are elevated in the serum of patients with RA. Based on these observations, CXCL13 could play an important role in the process of bone remodelling. Indeed, high CXCL13 levels were shown to be associated with more severe joint destruction over time in 2 Dutch

cohorts. This biomarker was most valuable in the anti–CCP-2–negative subpopulation of RA patients.[88]

IL2RA

IL2RA (CD25), the high affinity α chain of the IL2 receptor, is expressed on many immune cells and measurable in serum after cleavage from the membrane. It is considered a marker of T-cell proliferation. Several studies in other autoimmune diseases and healthy persons showed that the genetic variant described above (rs2104286) is associated with higher serum levels. In RA, higher serum levels are associated with more severe joint damage.[34] If this association is replicated in other cohorts, it could prove to be a relevant biomarker that is also more easily measurable than the genetic variant.

IMAGING BIOMARKERS AND THE SEVERITY OF RADIOGRAPHIC JOINT DESTRUCTION IN RA

The most frequently investigated imaging biomarkers for predicting radiographic joint destruction in RA patients are markers that are visible with MRI and ultrasound, namely, bone marrow edema, synovitis, tenosynovitis, and erosions.

MRI

MRI is increasingly used to measure disease states and treatment response in RA research. MRI has important advantages over conventional radiographs; in particular, it makes it possible to visualize and quantify inflammation of synovium, tendons, and bone (bone marrow edema), as well as structural damage. Bone marrow edema is common in RA and is estimated to occur in 68%-75% of patients with early RA.[89] Bone marrow edema is not detected by ultrasound or other imaging modalities and is a strong predictor of erosive progression. In a randomized controlled trial consisting of 130 RA patients, Hetland et al showed that bone marrow edema is an independent predictor of 2-year radiographic progression (coefficient, 0.59-0.75; $P<0.001$; $R^2=25\%-41\%$). Bone marrow edema also predicted radiographic progression at 5 years (coefficient, 0.83; $P<0.001$; $R^2=23\%$).[90;91] In an observational cohort of 84 early RA patients, Boyesen and Haavardsholm et al identified baseline bone marrow edema as an independent predictor of both 1-year radiographic erosive progression (OR=2.8, $P=0.04$) and 1-year MRI erosive progression (OR=1.3; $P=0.04$).[92-94] In an observational cohort of 42 patients, McQueen et al also demonstrated that 1-year radiographic erosions were more frequent in patients who had a total MRI score at baseline > 13 (OR=12.4; $P=0.002$)[95]. Additionally, they followed 31 of these patients over 6 years and observed that bone marrow edema at baseline also predicted the severity of radiographic joint destruction after 6 years ($R^2=0.20$; $P=.01$).[96] In an observational cohort of 24 early RA

patients, Lindegaard et al showed an RR of 4.0 for 1-year radiographic erosion when bone marrow edema was observed on the MRI; when erosion was observed on the MRI scan, the RR was 12.1.[97] In an observational cohort of 40 early RA patients, Conaghan et al showed a clear relationship between baseline MRI synovitis and development of subsequent MRI erosive destruction (area under the curve for MRI synovitis, r=0.42, $P<0.007$).[98] However, they did not assess the relationship with radiographic destruction. Boyesen et al showed that baseline synovitis on MRI independently predicted 3-year radiographic progression (beta=0.14; $P=0.03$).[92] Altogether, these studies confirm that the presence of synovitis and bone marrow edema on MRI is predictive of radiographic progression in patients with early RA. Again, the number of patients included in these studies was relatively low, and the observed effect sizes large.

An association between inflammation markers on MRI and radiographic joint destruction cannot always be identified. This can in part be explained by the short follow-up; assuming that bone marrow edema is a pre-erosive lesion, it takes time for a lesion to evolve from osteitis to erosion. Hoving et al observed that only half of the patients with erosions on MRI at baseline progressed to erosions on radiographs after a follow-up of 6 months.[99] Kamishima et al did not find a significant correlation between bone marrow edema and 1-year radiographic progression in 29 RA patients treated with anti-interleukin 6 receptor antibody. However, they did find a significant correlation between erosion on MRI and 1-year radiographic progression.[100] Furthermore, Ostergaard et al showed that MRI is considerably more sensitive than conventional radiographs; most new radiographic erosions (78%) were visualized at least 1 year earlier by MRI than by conventional radiograph, and MRI detection of new radiographic erosions preceded radiographic detection by a median of 2 years.[101] In a sample of 16 RA patients, Scheel et al showed that 41% of the erosions on MRI at baseline were seen on the radiograph at 7 years.[102] Although not every bone edema lesion evolves to erosion on the radiograph, most studies show that the development of radiographic erosions in the short term was highly unlikely in the absence of baseline MRI inflammatory changes. McQueen et al found a positive predictive value of 0.53 and a negative predictive value of 0.92 for bone marrow edema at baseline and radiographic erosions at 1 year.[95] Mundwiler et al reported similar findings only in the metatarsophalangeal joints of RA patients.[103]

Ultrasound

Although MRI is a potentially powerful technique for evaluation of inflammation and structural damage in RA, it is not a routine procedure owing to limited availability and high costs. Ultrasound is more available, less expensive, and does not require administration of contrast medium. It can be used to evaluate inflammation of joints and tendons and erosions. Synovitis is usually scored semi-quantitatively for both gray scale synovitis and power Doppler activity.

In an observational cohort of 84 RA patients, Boyesen et al showed that ultrasound gray-scale inflammation predicted 1-year MRI erosive progression (OR=2.01, p=0.02).[92] In their study of 59 RA patients starting anti-TNF therapy, Dougados et al. showed that baseline synovitis increased the risk of structural radiographic progression. The results of this study also implied that ultrasonographic examinations are not superior to clinical examination for predicting structural radiographic progression in RA.[104]

Tenosynovitis of the extensor carpi ulnaris was an imaging biomarker with an independent predictor for MRI erosive progression in the cohort of Lillegraven et al.[105]

Early RA bone erosions are detected with greater sensitivity using ultrasound than using conventional radiography, most probably as a direct function of their size. Szkudlarek et al. found more and larger erosions in the finger and toe joints of patients with established RA than in early RA.[106] In their study of 16 RA patients, Scheel et al showed that 22% of the erosions on ultrasound at baseline were seen on radiograph at 7 years; this percentage was lower than that detected by MRI.[102]

Ultrasound is limited by its capacity to detect lesions at some locations, such as the wrist and intra-articular surfaces of the third and fourth metacarpophalangeal joints. In addition, discrimination between cortical irregularity and erosions is problematic. Furthermore, with ultrasound, it is not possible to visualize inflammation of the bone (bone marrow edema). Ultrasound is also highly operator-dependent and time-consuming.

More research is necessary before ultrasound can be considered a useful tool for predicting radiographic progression of joint damage in RA.

COMBINING BIOMARKERS

Treatment of RA is not adjusted to individual prognoses but to measured disease activity. Consequently, some RA patients may be undertreated if disease activity is not suitably suppressed (generally patients with severe disease). Undertreatment could be attributed to the fact that aggressive combination therapy or biologics are not universally prescribed because of costs and concerns over toxicity. This reasoning underlines the need for a good prediction metric to identify patients with a potentially severe disease course. Although several prediction models or risk matrices have been developed, none have been validated. Furthermore, these models adequately predicted outcome in only about 50% of patients. Better models are necessary.

The existing models for evaluation of the severity of the course of RA are based on CRP, ACPA titer, baseline erosions, and the number of swollen joints.[69,107-110] This review of biomarkers of progression in RA shows that most of the biomarkers identified had not yet been integrated in prediction models. The addition of more recently identified genetic, serologic, and imaging markers will increase predictive ability.

CONCLUSION

Progression of joint destruction is an objective measure of the severity of RA and is frequently used to identify biomarkers that can be used to evaluate disease course. The highest sensitivity is observed with hand and foot radiographs taken serially over time and scored using a validated quantitative scoring method. Precise measurements increase statistical power, which is very relevant in genetic studies, since most genetic factors have small effect sizes. Serologic and imaging factors generally have larger effect sizes, probably because they are more closely related to the phenotype (Figure 3). The present manuscript provides an overview of genetic markers of severity studied in multiple cohorts; several markers for which a positive association was established in older studies were not replicated in more recent studies. For some markers, no definite conclusion could be drawn, thus necessitating a meta-analysis. Nonetheless, more than 10 genetic risk factors have been identified and replicated. Furthermore, various serologic and imaging risk factors were described. Most of these known risk factors have not yet been included in risk models. Combination of these markers to achieve adequate predictive value requires further study.

REFERENCE LIST

1. van der Helm-van Mil AH, Knevel R, van der Heijde D, et al. How to avoid phenotypic misclassification in using joint destruction as an outcome measure for rheumatoid arthritis? *Rheumatology (Oxford)* 2010 Aug;49(8):1429-35.
2. Welsing PM, van Gestel AM, Swinkels HL, et al. The relationship between disease activity, joint destruction, and functional capacity over the course of rheumatoid arthritis. *Arthritis Rheum* 2001 Sep;44(9):2009-17.
3. Landewe R, van der Heijde D, Klareskog L, et al. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. *Arthritis Rheum* 2006 Oct;54(10):3119-25.
4. Combe B, Cantagrel A, Goupille P, et al. Predictive factors of 5-year health assessment questionnaire disability in early rheumatoid arthritis. *J Rheumatol* 2003 Nov;30(11):2344-9.
5. Combe B. Progression in early rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2009 Feb;23(1):59-69.
6. Bathon J, Robles M, Ximenes AC, et al. Sustained disease remission and inhibition of radiographic progression in methotrexate-naive patients with rheumatoid arthritis and poor prognostic factors treated with abatacept: 2-year outcomes. *Ann Rheum Dis* 2011 Nov;70(11):1949-56.
7. Sanmarti R, Gomez A, Ercilla G, et al. Radiological progression in early rheumatoid arthritis after DMARDS: a one-year follow-up study in a clinical setting. *Rheumatology (Oxford)* 2003 Sep;42(9):1044-9.
8. Rau R, Herborn G, Menninger H, et al. Radiographic outcome after three years of patients with early erosive rheumatoid arthritis treated with intramuscular methotrexate or parenteral gold. Extension of a one-year double-blind study in 174 patients. *Rheumatology (Oxford)* 2002 Feb;41(2):196-204.
9. Nell-Duxneuner V, Machold K, Stamm T, et al. Autoantibody profiling in patients with very early rheumatoid arthritis: a follow-up study. *Ann Rheum Dis* 2010 Jan;69(1):169-74.
10. Sharp JT, Tsuji W, Ory P, et al. Denosumab prevents metacarpal shaft cortical bone loss in patients with erosive rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2010 Apr;62(4):537-44.
11. Knevel R, Lukas C, van der Heijde D, et al. Defining erosive disease typical of RA in the light of the ACR/EULAR 2010 criteria for rheumatoid arthritis; results of the data driven phase. *Ann Rheum Dis* 2013 Apr;72(4):590-5.
12. van der Heijde DM. Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability. *Baillieres Clin Rheumatol* 1996 Aug;10(3):435-53.
13. Larsen A. The value of individual joints for radiologic assessment of rheumatoid arthritis. *Scand J Rheumatol* 1976;5(2):119-23.
14. Knevel R, Tsonaka R, le Cessie S, et al. Comparison of methodologies for analysing the progression of joint destruction in rheumatoid arthritis. *Scand J Rheumatol* 2013;42(3):182-9.
15. Lindqvist E, Jonsson K, Saxne T, et al. Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. *Ann Rheum Dis* 2003 Jul;62(7):611-6.
16. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001 Mar;69(3):89-95.
17. Syversen SW, Landewe R, van der Heijde D, et al. Testing of the OMERACT 8 draft validation criteria for a soluble biomarker reflecting structural damage in rheumatoid arthritis: a systematic literature search on 5 candidate biomarkers. *J Rheumatol* 2009 Aug;36(8):1769-84.
18. Knevel R, Grondal G, Huizinga TW, et al. Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. *Ann Rheum Dis* 2012 May;71(5):707-9.

19. Too CL, Yahya A, Murad S, et al. Smoking interacts with HLA-DRB1 shared epitope in the development of anti-citrullinated protein antibody-positive rheumatoid arthritis: results from the Malaysian Epidemiological Investigation of Rheumatoid Arthritis (MyEIRA). *Arthritis Res Ther* 2012;14(2):R89.
20. Gregersen PK. Susceptibility genes for rheumatoid arthritis - a rapidly expanding harvest. *Bull NYU Hosp Jt Dis* 2010;68(3):179-82.
21. van der Helm-van Mil AH, Huizinga TW, Schreuder GM, et al. An independent role of protective HLA class II alleles in rheumatoid arthritis severity and susceptibility. *Arthritis Rheum* 2005 Sep;52(9):2637-44.
22. Lie BA, Viken MK, Odegard S, et al. Associations between the PTPN22 1858C->T polymorphism and radiographic joint destruction in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 2007 Dec;66(12):1604-9.
23. Marinou I, Healy J, Mewar D, et al. Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. *Arthritis Rheum* 2007 Aug;56(8):2549-56.
24. Karlson EW, Chibnik LB, Cui J, et al. Associations between human leukocyte antigen, PTPN22, CTLA4 genotypes and rheumatoid arthritis phenotypes of autoantibody status, age at diagnosis and erosions in a large cohort study. *Ann Rheum Dis* 2008 Mar;67(3):358-63.
25. Pierer M, Kaltenhauser S, Arnold S, et al. Association of PTPN22 1858 single-nucleotide polymorphism with rheumatoid arthritis in a German cohort: higher frequency of the risk allele in male compared to female patients. *Arthritis Res Ther* 2006;8(3):R75.
26. Steer S, Lad B, Grumley JA, et al. Association of R602W in a protein tyrosine phosphatase gene with a high risk of rheumatoid arthritis in a British population: evidence for an early onset/disease severity effect. *Arthritis Rheum* 2005 Jan;52(1):358-60.
27. van Nies JA, Knevel R, Daha N, et al. The PTPN22 susceptibility risk variant is not associated with the rate of joint destruction in anti-citrullinated protein antibody-positive rheumatoid arthritis. *Ann Rheum Dis* 2010 Sep;69(9):1730-1.
28. Maxwell JR, Marinou I, Kuet KP, et al. Rheumatoid arthritis-associated polymorphisms at 6q23 are associated with radiological damage in autoantibody-positive RA. *J Rheumatol* 2012 Sep;39(9):1781-5.
29. Scherer HU, van der Linden MP, Kurreeman FA, et al. Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis. *Ann Rheum Dis* 2010 Mar;69(3):567-70.
30. Knevel R, de Rooy DP, Gregersen PK, et al. Studying associations between variants in TRAF1-C5 and TNFAIP3-OLIG3 and the progression of joint destruction in rheumatoid arthritis in multiple cohorts. *Ann Rheum Dis* 2012 Oct;71(10):1753-5.
31. Viatte S, Plant D, Lunt M, et al. Investigation of rheumatoid arthritis genetic susceptibility markers in the early rheumatoid arthritis study further replicates the TRAF1 association with radiological damage. *J Rheumatol* 2013 Feb;40(2):144-56.
32. Kurreeman FA, Padyukov L, Marques RB, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. *PLoS Med* 2007 Sep;4(9):e278.
33. van der Linden MP, Feitsma AL, le Cessie S, et al. Association of a single-nucleotide polymorphism in CD40 with the rate of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2009 Aug;60(8):2242-7.
34. Knevel R, de Rooy DP, Zhernakova A, et al. Association of variants in IL2RA with progression of joint destruction in Rheumatoid Arthritis. *Arthritis Rheum* 2013 Mar 25.
35. Kleyer A, Finzel S, Rech J, et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann Rheum Dis* 2013 Mar 21.
36. Genevay S, Di Giovine FS, Perneger TV, et al. Association of interleukin-4 and interleukin-1B gene variants with Larsen score progression in rheumatoid arthritis. *Arthritis Rheum* 2002 Jun 15;47(3):303-9.

37. Johnsen AK, Plenge RM, Butty V, et al. A broad analysis of IL1 polymorphism and rheumatoid arthritis. *Arthritis Rheum* 2008 Jul;58(7):1947-57.
38. Buchs N, Di Giovine FS, Silvestri T, et al. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. *Genes Immun* 2001 Jun;2(4):222-8.
39. Marotte H, Farge P, Gaudin P, et al. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Ann Rheum Dis* 2006 Jul;65(7):905-9.
40. Prots I, Skapenko A, Wendler J, et al. Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006 May;54(5):1491-500.
41. Krabben A, Wilson AG, de Rooy DP, et al. Brief Report: Association of Genetic Variants in the IL4 and IL4R Genes With the Severity of Joint Damage in Rheumatoid Arthritis: A Study in Seven Cohorts. *Arthritis Rheum* 2013 Dec;65(12):3051-7.
42. Marinou I, Till SH, Moore DJ, et al. Lack of association or interactions between the IL-4, IL-4Ralpha and IL-13 genes, and rheumatoid arthritis. *Arthritis Res Ther* 2008;10(4):R80.
43. Huizinga TW, Keijsers V, Yanni G, et al. Are differences in interleukin 10 production associated with joint damage? *Rheumatology (Oxford)* 2000 Nov;39(11):1180-8.
44. Cantagrel A, Navaux F, Loubet-Lescoulie P, et al. Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum* 1999 Jun;42(6):1093-100.
45. Pawlik A, Kurzawski M, Szklarz BG, et al. Interleukin-10 promoter polymorphism in patients with rheumatoid arthritis. *Clin Rheumatol* 2005 Sep;24(5):480-4.
46. Knevel R, Krabben A, Brouwer E, et al. Genetic variants in IL15 associate with progression of joint destruction in rheumatoid arthritis: a multicohort study. *Ann Rheum Dis* 2012 Oct;71(10):1651-7.
47. Han SW, Sa KH, Kim SI, et al. FCRL3 gene polymorphisms contribute to the radiographic severity rather than susceptibility of rheumatoid arthritis. *Hum Immunol* 2012 May;73(5):537-42.
48. Maehlen MT, Nordang GB, Syversen SW, et al. FCRL3 -169C/C genotype is associated with anti-citrullinated protein antibody-positive rheumatoid arthritis and with radiographic progression. *J Rheumatol* 2011 Nov;38(11):2329-35.
49. Sugiura Y, Niimi T, Sato S, et al. Transforming growth factor beta1 gene polymorphism in rheumatoid arthritis. *Ann Rheum Dis* 2002 Sep;61(9):826-8.
50. Kim SY, Han SW, Kim GW, et al. TGF-beta1 polymorphism determines the progression of joint damage in rheumatoid arthritis. *Scand J Rheumatol* 2004;33(6):389-94.
51. Mattey DL, Nixon N, Dawes PT, et al. Association of polymorphism in the transforming growth factor beta 1 gene with disease outcome and mortality in rheumatoid arthritis. *Ann Rheum Dis* 2005 Aug;64(8):1190-4.
52. de Rooy DP, Yeremenko NG, Wilson AG, et al. Genetic studies on components of the Wnt signalling pathway and the severity of joint destruction in rheumatoid arthritis. *Ann Rheum Dis* 2013 May;72(5):769-75.
53. Knevel R, Krabben A, Wilson AG, et al. A genetic variant in granzyme B is associated with progression of joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2013 Mar;65(3):582-9.
54. Stanton H, Rogerson FM, East CJ, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature* 2005 Mar 31;434(7033):648-52.
55. Lohmander LS, Neame PJ, Sandy JD. The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis. *Arthritis Rheum* 1993 Sep;36(9):1214-22.

56. Glasson SS, Askew R, Sheppard B, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 2005 Mar 31;434(7033):644-8.
57. Constantin A, Lauwers-Cances V, Navaux F, et al. Stromelysin 1 (matrix metalloproteinase 3) and HLA-DRB1 gene polymorphisms: Association with severity and progression of rheumatoid arthritis in a prospective study. *Arthritis Rheum* 2002 Jul;46(7):1754-62.
58. Mattey DL, Nixon NB, Dawes PT, et al. Association of matrix metalloproteinase 3 promoter genotype with disease outcome in rheumatoid arthritis. *Genes Immun* 2004 Mar;5(2):147-9.
59. Dorr S, Lechtenbohmer N, Rau R, et al. Association of a specific haplotype across the genes MMP1 and MMP3 with radiographic joint destruction in rheumatoid arthritis. *Arthritis Res Ther* 2004;6(3):R199-R207.
60. de Rooy DP, Zhernakova A, Tsonaka R, et al. A genetic variant in the region of MMP-9 is associated with serum levels and progression of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2013 May 21.
61. Knevel R, Klein K, Somers K, et al. Identification of a genetic variant for joint damage progression in autoantibody-positive rheumatoid arthritis. *Ann Rheum Dis* 2013 Aug 16.
62. Bukhari M, Thomson W, Naseem H, et al. The performance of anti-cyclic citrullinated peptide antibodies in predicting the severity of radiologic damage in inflammatory polyarthritis: results from the Norfolk Arthritis Register. *Arthritis Rheum* 2007 Sep;56(9):2929-35.
63. Courvoisier N, Dougados M, Cantagrel A, et al. Prognostic factors of 10-year radiographic outcome in early rheumatoid arthritis: a prospective study. *Arthritis Res Ther* 2008;10(5):R106.
64. Drossaers-Bakker KW, Zwinderman AH, Vliet Vlieland TP, et al. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. *Arthritis Rheum* 2002 Aug;47(4):383-90.
65. Forslind K, Ahlmen M, Eberhardt K, et al. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* 2004 Sep;63(9):1090-5.
66. Kaltenhauser S, Wagner U, Schuster E, et al. Immunogenetic markers and seropositivity predict radiological progression in early rheumatoid arthritis independent of disease activity. *J Rheumatol* 2001 Apr;28(4):735-44.
67. Lindqvist E, Eberhardt K, Bendtzen K, et al. Prognostic laboratory markers of joint damage in rheumatoid arthritis. *Ann Rheum Dis* 2005 Feb;64(2):196-201.
68. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. *Arthritis Res Ther* 2006;8(4):R128.
69. Syversen SW, Gaarder PI, Goll GL, et al. High anti-cyclic citrullinated peptide levels and an algorithm of four variables predict radiographic progression in patients with rheumatoid arthritis: results from a 10-year longitudinal study. *Ann Rheum Dis* 2008 Feb;67(2):212-7.
70. Vencovsky J, Machacek S, Sedova L, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. *Ann Rheum Dis* 2003 May;62(5):427-30.
71. van der Linden MP, van der Woude D, Ioan-Facsinay A, et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. *Arthritis Rheum* 2009 Aug;60(8):2232-41.
72. Harris ML, Darrah E, Lam GK, et al. Association of autoimmunity to peptidyl arginine deiminase type 4 with genotype and disease severity in rheumatoid arthritis. *Arthritis Rheum* 2008 Jul;58(7):1958-67.
73. Shi J, Knevel R, Suwannalai P, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid

arthritis and predict joint damage. *Proc Natl Acad Sci U S A* 2011 Oct 18;108(42):17372-7.

74. Knevel R, van Nies JA, le Cessie S, et al. Evaluation of the contribution of cumulative levels of inflammation to the variance in joint destruction in rheumatoid arthritis. *Ann Rheum Dis* 2013 Feb;72(2):307-8.
75. Tchetverikov I, Lard LR, DeGroot J, et al. Matrix metalloproteinases-3, -8, -9 as markers of disease activity and joint damage progression in early rheumatoid arthritis. *Ann Rheum Dis* 2003 Nov;62(11):1094-9.
76. Garnero P, Gineyts E, Christgau S, et al. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. *Arthritis Rheum* 2002 Jan;46(1):21-30.
77. Jansen LM, van dH-B, I, Lems WF, et al. Serological bone markers and joint damage in early polyarthritis. *J Rheumatol* 2004 Aug;31(8):1491-6.
78. Le Loët X, Brazier M, Mejjad O, et al. Serum IgA rheumatoid factor and pyridinoline in very early arthritis as predictors of erosion(s) at two years: a simple model of prediction from a conservatively treated community-based inception cohort. *Arthritis Care Res (Hoboken)* 2010 Dec;62(12):1739-47.
79. Young-Min S, Cawston T, Marshall N, et al. Biomarkers predict radiographic progression in early rheumatoid arthritis and perform well compared with traditional markers. *Arthritis Rheum* 2007 Oct;56(10):3236-47.
80. den Broeder AA, Joosten LA, Saxne T, et al. Long term anti-tumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis* 2002 Apr;61(4):311-8.
81. Roux-Lombard P, Eberhardt K, Saxne T, et al. Cytokines, metalloproteinases, their inhibitors and cartilage oligomeric matrix protein: relationship to radiological progression and inflammation in early rheumatoid arthritis. A prospective 5-year study. *Rheumatology (Oxford)* 2001 May;40(5):544-51.
82. Garnero P, Landewe R, Boers M, et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. *Arthritis Rheum* 2002 Nov;46(11):2847-56.
83. Jensen T, Klarlund M, Hansen M, et al. Connective tissue metabolism in patients with unclassified polyarthritis and early rheumatoid arthritis. Relationship to disease activity, bone mineral density, and radiographic outcome. *J Rheumatol* 2004 Sep;31(9):1698-708.
84. Krabben A, Knevel R, Huizinga TW, et al. Serum pyridinoline levels and prediction of severity of joint destruction in rheumatoid arthritis. *J Rheumatol.* In press 2013.
85. Skoumal M, Kolarz G, Woloszczuk W, et al. Serum osteoprotegerin but not receptor activator of NF-kappaB ligand correlates with Larsen score in rheumatoid arthritis. *Ann Rheum Dis* 2004 Feb;63(2):216-7.
86. Geusens PP, Landewe RB, Garnero P, et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006 Jun;54(6):1772-7.
87. van Tuyl LH, Voskuyl AE, Boers M, et al. Baseline RANKL:OPG ratio and markers of bone and cartilage degradation predict annual radiological progression over 11 years in rheumatoid arthritis. *Ann Rheum Dis* 2010 Sep;69(9):1623-8.
88. Meeuwisse CM, van der Linden MP, Rullmann TA, et al. Identification of CXCL13 as a marker for rheumatoid arthritis outcome using an in silico model of the rheumatic joint. *Arthritis Rheum* 2011 May;63(5):1265-73.
89. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals a high prevalence of erosions at four months after symptom onset. *Ann Rheum Dis* 1998 Jun;57(6):350-6.
90. Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in

early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009 Mar;68(3):384-90.

91. Hetland ML, Stengaard-Pedersen K, Junker P, et al. Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 2010 Oct;69(10):1789-95.
92. Boyesen P, Haavardsholm EA, van der Heijde D, et al. Prediction of MRI erosive progression: a comparison of modern imaging modalities in early rheumatoid arthritis patients. *Ann Rheum Dis* 2011 Jan;70(1):176-9.
93. Boyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011 Mar;70(3):428-33.
94. Haavardsholm EA, Boyesen P, Ostergaard M, et al. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. *Ann Rheum Dis* 2008 Jun;67(6):794-800.
95. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 1999 Mar;58(3):156-63.
96. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003 Jul;48(7):1814-27.
97. Lindegaard HM, Vallo J, Horslev-Petersen K, et al. Low-cost, low-field dedicated extremity magnetic resonance imaging in early rheumatoid arthritis: a 1-year follow-up study. *Ann Rheum Dis* 2006 Sep;65(9):1208-12.
98. Conaghan PG, O'Connor P, McGonagle D, et al. Elucidation of the relationship between synovitis and bone damage: a randomized magnetic resonance imaging study of individual joints in patients with early rheumatoid arthritis. *Arthritis Rheum* 2003 Jan;48(1):64-71.
99. Hoving JL, Buchbinder R, Hall S, et al. A comparison of magnetic resonance imaging, sonography, and radiography of the hand in patients with early rheumatoid arthritis. *J Rheumatol* 2004 Apr;31(4):663-75.
100. Kamishima T, Tanimura K, Shimizu M, et al. Monitoring anti-interleukin 6 receptor antibody treatment for rheumatoid arthritis by quantitative magnetic resonance imaging of the hand and power Doppler ultrasonography of the finger. *Skeletal Radiol* 2011 Jun;40(6):745-55.
101. Ostergaard M, Hansen M, Stoltenberg M, et al. New radiographic bone erosions in the wrists of patients with rheumatoid arthritis are detectable with magnetic resonance imaging a median of two years earlier. *Arthritis Rheum* 2003 Aug;48(8):2128-31.
102. Scheel AK, Hermann KG, Ohrndorf S, et al. Prospective 7 year follow up imaging study comparing radiography, ultrasonography, and magnetic resonance imaging in rheumatoid arthritis finger joints. *Ann Rheum Dis* 2006 May;65(5):595-600.
103. Mundwiler ML, Maranian P, Brown DH, et al. The utility of MRI in predicting radiographic erosions in the metatarsophalangeal joints of the rheumatoid foot: a prospective longitudinal cohort study. *Arthritis Res Ther* 2009;11(3):R94.
104. Dougados M, Devauchelle-Pensec V, Ferlet JF, et al. The ability of synovitis to predict structural damage in rheumatoid arthritis: a comparative study between clinical examination and ultrasound. *Ann Rheum Dis* 2013 May;72(5):665-71.
105. Lillegraven S, Boyesen P, Hammer HB, et al. Tenosynovitis of the extensor carpi ulnaris tendon predicts erosive progression in early rheumatoid arthritis. *Ann Rheum Dis* 2011 Nov;70(11):2049-50.
106. Szkudlarek M, Narvestad E, Klarlund M, et al. Ultrasonography of the metatarsophalangeal joints in rheumatoid arthritis: comparison with magnetic resonance imaging, conventional

radiography, and clinical examination. *Arthritis Rheum* 2004 Jul;50(7):2103-12.

107. Fautrel B, Granger B, Combe B, et al. Matrix to predict rapid radiographic progression of early rheumatoid arthritis patients from the community treated with methotrexate or leflunomide: results from the ESPOIR cohort. *Arthritis Res Ther* 2012 Nov 19;14(6):R249.
108. Lillegraven S, Paynter N, Prince FH, et al. Performance of matrix-based risk models for rapid radiographic progression in a cohort of patients with established rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2013 Apr;65(4):526-33.
109. Vastesaeger N, Xu S, Aletaha D, et al. A pilot risk model for the prediction of rapid radiographic progression in rheumatoid arthritis. *Rheumatology (Oxford)* 2009 Sep;48(9):1114-21.
110. Visser K, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, et al. A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the BeSt study. *Ann Rheum Dis* 2010 Jul;69(7):1333-7.
111. Dadoun S, Zeboulon-Ktorza N, Combescure C, et al. Mortality in rheumatoid arthritis over the last fifty years: systematic review and meta-analysis. *Joint Bone Spine* 2013 Jan;80(1):29-33.
112. van Nies JA, de Jong Z, van der Helm-van Mil AH, et al. Improved treatment strategies reduce the increased mortality risk in early RA patients. *Rheumatology (Oxford)* 2010 Nov;49(11):2210-6.
113. van der Woude D, Young A, Jayakumar K, et al. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. *Arthritis Rheum* 2009 Aug;60(8):2262-71.
114. Goronzy JJ, Matteson EL, Fulbright JW, et al. Prognostic markers of radiographic progression in early rheumatoid arthritis. *Arthritis Rheum* 2004 Jan;50(1):43-54.
115. Knevel R, de Rooy DP, Gregersen PK, et al. Studying associations between variants in TRAF1-C5 and TNFAIP3-OLIG3 and the progression of joint destruction in rheumatoid arthritis in multiple cohorts. *Ann Rheum Dis* 2012 Oct;71(10):1753-5.
116. Green MJ, Gough AK, Devlin J, et al. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology (Oxford)* 2003 Jan;42(1):83-8.
117. Yamanaka H, Matsuda Y, Tanaka M, et al. Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. *Arthritis Rheum* 2000 Apr;43(4):852-8.
118. Posthumus MD, Limburg PC, Westra J, et al. Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. *Rheumatology (Oxford)* 1999 Nov;38(11):1081-7.

5

Genetic variants in *IL15* associate with progression of joint destruction in rheumatoid arthritis: a multicohort study

R. Knevel, A. Krabben, E. Brouwer, M. D. Posthumus,
A. G. Wilson, E. Lindqvist, T. Saxne, D. P. C. de Rooy,
N. A. Daha, M. P. M. van der Linden, G. Stoeken, L. van Toorn,
B. Koeleman, R. Tsonaka, A. Zhernakoza,
J. J. Houwing-Duistermaat, R. E. M. Toes, T. W. J. Huizinga,
A. H. M. van der Helm-van Mil

Annals of the Rheumatic Diseases, 2012 October;71(10):1651–1657.

ABSTRACT

Background

Interleukin (IL)-15 levels are increased in serum, synovium and bone marrow of patients with rheumatoid arthritis (RA). IL-15 influences both the innate and the adaptive immune response; its major role is activation and proliferation of T cells. There are also emerging data that IL-15 affects osteoclastogenesis. The authors investigated the association of genetic variants in *IL15* with the rate of joint destruction in RA.

Method

1418 patients with 4885 x-ray sets of both hands and feet of four independent data sets were studied. First, explorative analyses were performed on 600 patients with early RA enrolled in the Leiden Early Arthritis Clinic. Twenty-five single-nucleotide polymorphisms (SNPs) tagging *IL15* were tested. Second, SNPs with significant associations in the explorative phase were genotyped in data sets from Groningen, Sheffield and Lund. In each data set, the relative increase of the progression rate per year in the presence of a genotype was assessed. Subsequently, data were summarized in an inverse weighting meta-analysis.

Results

Five SNPs were significantly associated with rate of joint destruction in phase 1 and typed in the other data sets. Patients homozygous for rs7667746, rs7665842, rs2322182, rs6821171 and rs4371699 had respectively 0.94-, 1.04-, 1.09-, 1.09- and 1.09-fold rate of joint destruction compared to other patients ($p=4.0\times10^{-6}$, $p=3.8\times10^{-4}$, $p=5.0\times10^{-3}$, $p=5.0\times10^{-3}$ and $p=9.4\times10^{-3}$).

Discussion

Independent replication was not obtained, possibly due to insufficient power. Meta-analyses of all data sets combined resulted in significant results for four SNPs (rs7667746, $p<0.001$; rs7665842, $p<0.001$; rs4371699, $p=0.01$; rs6821171, $p=0.01$). These SNPs were also significant after correction for multiple testing.

Conclusion

Genetic variants in IL-15 are associated with progression of joint destruction in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that affects 0.5–1% of the population and is associated with significant morbidity, disability and costs for society. Radiographic joint destruction reflects the cumulative burden of inflammation and is conceived as an objective measure of RA severity. The degree of joint destruction varies significantly between patients. The processes behind this difference are incompletely understood. Inflammatory markers and autoantibodies are potent risk factors for joint destruction but account for approximately 30% of the variance in joint destruction.[1] Recent data indicate that genetic factors influence the severity of joint destruction in RA.[2] Hence, to increase the understanding of progression mediating disease processes, it is relevant to study genetic variants that could predispose for a severe outcome of RA.

In this article, we describe a candidate gene study to investigate the association of interleukin (IL)-15 with the rate of joint destruction. IL-15 is expressed primarily by macrophages, as well as by fibroblast-like synoviocytes and endothelial cells.[3] IL-15 produced by synoviocytes has been implicated in the pathogenesis of RA as it serves as T lymphocyte activator and proliferator in the synovial membrane as well as in the bone marrow.[4-6] IL-15 may be implicated in the perpetuation of synovial inflammation by generating a positive feedback in which activated synovial macrophages or fibroblasts induce continuous T cell recruitment.[7] Results of several animal studies supported the notion that IL-15 has a role in RA progression. Ferrari-Lacraz *et al* generated a mutated IL-15 fused to the constant region of a murine IgG2a, which inhibited the IL-15 receptor. This mutated IL-15-IgG prevented the development of collagen-induced arthritis and also blocked disease progression in an established disease model.[8] Daily intraperitoneal injection of mutated IL-15-IgG in collagen-induced arthritis showed reduced clinical scores and reduced cartilage erosions relative to controls. Another study demonstrated that in the absence of IL-15 signaling, several converging mechanisms of osteoclastogenesis were inhibited in mice.[9] In patients with RA, IL-15 levels are increased in serum, synovial fluid and bone marrow.[4,6] Additionally, serum levels of IL-15 correlated strongly to disease activity.[10]

These data led us to hypothesize that genetic variants in *IL15* are associated with the severity of joint destruction in RA. We tested this hypothesis using four data sets of European patients with RA with longitudinal radiological data on joint destruction. All data sets included patients who were diagnosed in a period when treatment strategies were less aggressive and less controlled than today. These conservative treatment strategies made these data sets suitable for the present study as the natural course was less inhibited.

PATIENTS AND METHODS

Study population

Four data sets consisting of adult European patients with RA were studied. RA was defined according to the 1987 American College of Rheumatology criteria in all cohorts except for the Lund cohort where the 1958 criteria were used. X-rays of both hands and feet were available in all cohorts (table 1).

Table 1: Characteristics for each cohort.

Cohort	Leiden-EAC (n=600)	Groningen (n=275)	Sheffield (n=396)	Lund (n=147)
Year of diagnosis	1993-2006	1945-2001	1938-2003	1985-1989
Follow-up years*	7 years	14 years	Not applicable*	5 years
Total no. of X-ray sets	2,846	862	396	781
Method of scoring	SHS	SHS	Larsen	Larsen
Female n (%)	412 (69)	194 (71)	290 (73)	98 (67)
Age at diagnosis, mean ± SD	56 ± 16	49 ± 13	46 ± 13	51 ± 12
CCP+ n (%)	323 (55)	160 (80)	302 (79)	114 (80)

SHS= Sharp van-der-Heijde score

*Data of Leiden-EAC, Groningen and Lund were collected from baseline onwards during respectively 7, 14 and 5 years of follow-up. The data of Sheffield were collected once during the disease period, the mean disease duration was 15 years (range 3-65 years).

Leiden Early Arthritis Clinic cohort (Leiden-EAC)

This cohort concerned 600 patients with early RA from the western part of the Netherlands who were included in the Leiden-EAC between 1993 and 2006.[1] Patients were included at time of diagnosis and yearly followed up. X-rays were taken at baseline and on yearly follow-up visits for 7 years. In total, 2846 x-ray sets of hands and feet were available. All x-rays were chronologically scored by one experienced reader who was unaware of genetic or clinical data using Sharp–van der Heijde scores (SHSs) on hands and feet.[11] A total of 499 x-rays (belonging to 60 randomly selected patients with RA) were scored double. The correlation coefficient (ICC) within the reader was 0.91. The treatment of these patients could be divided into three treatment periods. Patients included in 1993–1995 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996–1998 were initially treated with chloroquine or salazopyrine and patients included after 1999 were promptly treated with methotrexate or salazopyrine.

Groningen

The second set of data involved 275 patients with RA from the northern part of the Netherlands who were diagnosed between 1945 and 2001. The follow-up duration after diagnosis was limited to 14 years. The mean number of x-ray sets (hands and feet) per patient was 3.1 (with a maximum of 8 x-rays per patient). The total number of sets of x-rays was 862. The x-rays were scored chronologically by one of two readers using SHS. ICCs within readers were >0.90, and those between readers were 0.96. The development of joint destruction was significantly different for patients included in the 1990s compared to patients included before 1990. This observation is in line with the introduction of treatment with disease-modifying anti-rheumatic drug (DMARD).

Sheffield

The third set of data concerned 396 patients with RA from the area of Sheffield, UK. Patients with RA with x-rays available were recruited from the rheumatology department of the Royal Hallamshire Hospital in Sheffield between 1999 and 2006 and were diagnosed as having RA between 1938 and 2003.[12] Patients with RA were assessed once during their disease course. The mean ± SD disease duration at assessment was 15±11 years (range: 3–65 years). X-rays of hands and feet were scored by one reader using a modification to Larsen's score.[13] Ten percent of films were scored twice to quantify the intra-observer variation by a weighted κ score, which was 0.83.[12]

Lund

This cohort concerned 183 Swedish patients with early RA who were prospectively followed up yearly for 5 years, of whom 147 had x-rays and DNA available.[14;15] Patients were recruited from primary care units in the area of Lund during 1985–1989. X-rays of hands and feet were taken at the start of the study and annually for 5 years, resulting in a total of 781 sets of x-rays. X-rays were scored chronologically according to Larsen by one of two readers.[16] The ICC between the readers determined on 105 x-rays was 0.94. In the inclusion period, immediate DMARD treatment was not common and only half of the patients used any DMARD at 5 years of follow-up, most commonly chloroquine, D-penicillamine, sodium aurothiomalate and auranofin.[14] All patients gave their informed consent, and approval was obtained from the local ethical committee of each study.

SNP selection and genotyping

The region of *IL15*, located at chromosome 4q31, plus the regions of the upstream- and downstream-situated haplotype blocks were tagged by the algorithm of haploview.[17] No coding and amino acid changing single-nucleotide polymorphisms (SNPs) are known for *IL15*. One SNP, rs12508866, had a significant association with RA susceptibility in the Wellcome Trust Case Control Consortium data set[18] and was therefore forced to be included.

Pairwise tagging SNPs were selected from the CEPH/ CEU hapmap data set (phase 2, release 21, NCBI (*National Center for Biotechnology Information*) build 35) using haploview software (minor allele frequency>0.05, pairwise r^2<0.8). A total of 25 SNPs captured all SNPs on *IL15*. Multiplex SNP arrays were designed using Illumina Golden Gate platform, according to the protocols recommended by the manufacturer (Illumina, San Diego, California, USA). Two SNPs (rs9884645 and rs4401531) were excluded as they could not be designed in the multiplex SNP array and no good proxy existed. The SNP selection and the linkage disequilibrium (LD) information are depicted in figure 1.

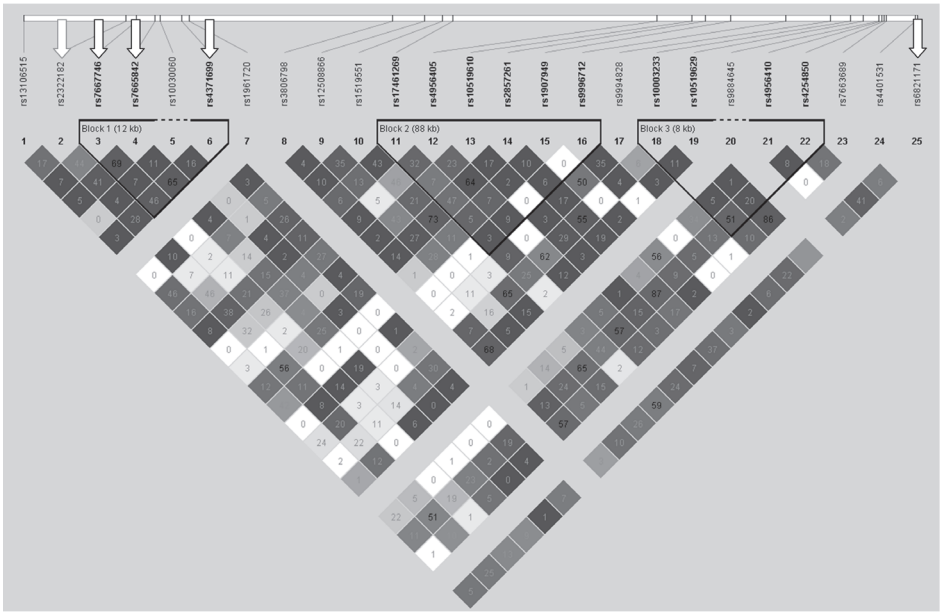


Figure 1 LD structure between of 25 tag-SNPs in IL-15.
The depicted data are from 600 Leiden-EAC Dutch RA-patients. The numbers present the r2 between the SNPs. The colours refer to D´. Two SNP could not be designed and no good proxy existed (rs9884645 no.20, rs4401531 no.24). One SNP failed typing (rs1961720, no.7). Significant SNPs in the analyses of Leiden-EAC are marked by an arrow.

Software supplied by Illumina was used to automatically identify the genotypes. Each 96-well plate consisted of one positive and one negative control. In all plates, the positive controls indeed tested positive and the negative controls tested negative. Clusters were evaluated and all doubtful calls were checked; after manually evaluating the spectra of each cluster, we accepted, recalled or rejected the genotypes. At least 12% were assessed in duplicate, with an error rate of <1% for all SNPs except rs7667746, which had an error rate of 3.8%. One SNP failed and two SNPs had a success rate of 75%; the remaining SNPs

were typed with a success rate of >98% (Supplementary Table 1). None of the SNPs were out of Hardy–Weinberg equilibrium ($p<0.001$).

SNPs that were significantly associated with joint destruction in the first data set were genotyped in the other three data sets. SNPs were genotyped with multiplex SNP arrays designed with Sequenom iPLEX, according to the protocols recommended by the manufacturer (Sequenom, San Diego, California, USA). Software supplied by the same manufacturer was used to automatically identify the genotypes. Each iPLEX consisted of at least nine positive and nine negative controls, which were indeed tested positive and negative. All doubtful calls were checked manually. DNA samples that still had >30% failed SNPs after manual checking were excluded from analysis (n=31). At least 5% of the genotypes were assessed in duplicate, with an error rate of <1%. The success rates were all >95%. No SNPs were out of Hardy–Weinberg equilibrium.

Statistical analysis

Associations between genotypes and radiographic joint destruction were analyzed. Two phases were carried out. First, an explorative analysis was performed in the Leiden-EAC. In this data set, the tagged SNPs were tested in two ways: additively and recessively. In all data sets, the radiological scores were log-transformed to obtain a normal distribution. Since phase 1 was an explorative phase, no correction for multiple testing was applied yet and SNPs with a p value ≤ 0.05 were studied in phase 2.

For the analyses in the Leiden-EAC, a multivariate normal regression model for longitudinal data was used with radiological score as response variable. This method analyses all repeated measurements at once and takes advantage of the correlation between these measurements. The effect of time was entered as a factor in the model, to properly capture the mean response profile over time. To test for an association with the rate of joint destruction, we conducted an analysis with the SNP and its interaction with time in the model. The effect of time in the interaction term was linear. Since the analyses were performed on the log scale, the resulting coefficient (β) on the original scale indicates how many fold the joint destruction increased per year in the tested genotype compared to reference genotype and increases per year by the power of the years of follow-up. Adjustment variables were entered based on their univariate association with joint destruction. Adjustments were made for age, gender and the described treatment periods.

For the analyses in Groningen and Lund, we used a multivariate normal regression analysis that was similar to the analysis applied in the Leiden-EAC. Adjustment variables were entered based on their univariate association with joint destruction. The Groningen data set was adjusted for age and inclusion before 1990 and after 1990, as proxy for DMARD treatment. The analysis of Lund was adjusted for age only, since gender and treatment were not associated with joint destruction in this data set.

In the Sheffield data set, each patient had x-rays at one time point. The estimated yearly progression rate was calculated to make the scores comparable to the other data sets.[19] This was achieved by dividing the total by the number of disease years at time of x. The disease duration at time of x-ray was available for 391 patients. The SNP association was tested in a linear regression analysis with log-transformed estimated yearly progression rate as outcome variable. No adjustments were applied as none of the tested variables was univariately significantly associated with joint destruction. Also, here, the resulting estimate reflects how many fold the rate of joint destruction increases per year in the presence of a minor allele compared to the absence of this allele. Analyses were performed using SPSS version 17.0.

In the present study, the power is the result of the number of patients and the number of measurements per patient studied. As shown previously, the precision of the estimate increases steadily with increasing numbers of x-rays per patient.[20] All three data sets studied to verify the results of phase 1 contained (individually and combined) less x-rays than the initial data set. Consequently, the power to replicate findings in each data set individually as well as in the three replication data sets together was limited in comparison to the large amount of x-rays in the discovery data set. Because of differences in study designs, the separate data sets could not be combined in one analysis directly. Therefore, we decided to test the SNPs in each data set separately, taking advantage of the specific data set characteristics, and to subsequently perform a meta-analysis on the results to determine the association of the SNPs with the rate of joint destruction. A fixed-effects meta-analysis[22] with inverse variance weighting was performed in Stata, version 10.1.

It is debatable whether correction for multiple testing should be applied. However, multiple testing correction using the Benjamini–Hochberg false discovery rate was performed in phase 2 to reduce the chance of having false-positive findings. p Values ≤ 0.05 after correction for multiple testing were considered significant.[23]

Haplotype analyses

Haplotypes of *IL15* were studied. Haplotype blocks were defined by Gabriel's method.[24] Haplotypes were assigned to each individual using PLINK 1.06 requiring a probability >0.8. Analyses of the haplotypes were performed with methods similar to those used for the analyses of the individual SNPs by now testing the presence of a haplotype compared to the absence of the haplotype.

RESULTS

Discovery phase

IL15 was tagged by 25 SNPs, of which 2 could not be designed, 1 failed typing and 2 did not pass quality control. In total, 20SNPs were analyzed in the Leiden-EAC. Five SNPs were significantly associated with joint destruction (minor allele): rs2322182(A), rs7667746(G), rs7665842(G), rs4371699(A) and rs6821171(C) (figure 2). The recessive analyses fitted the

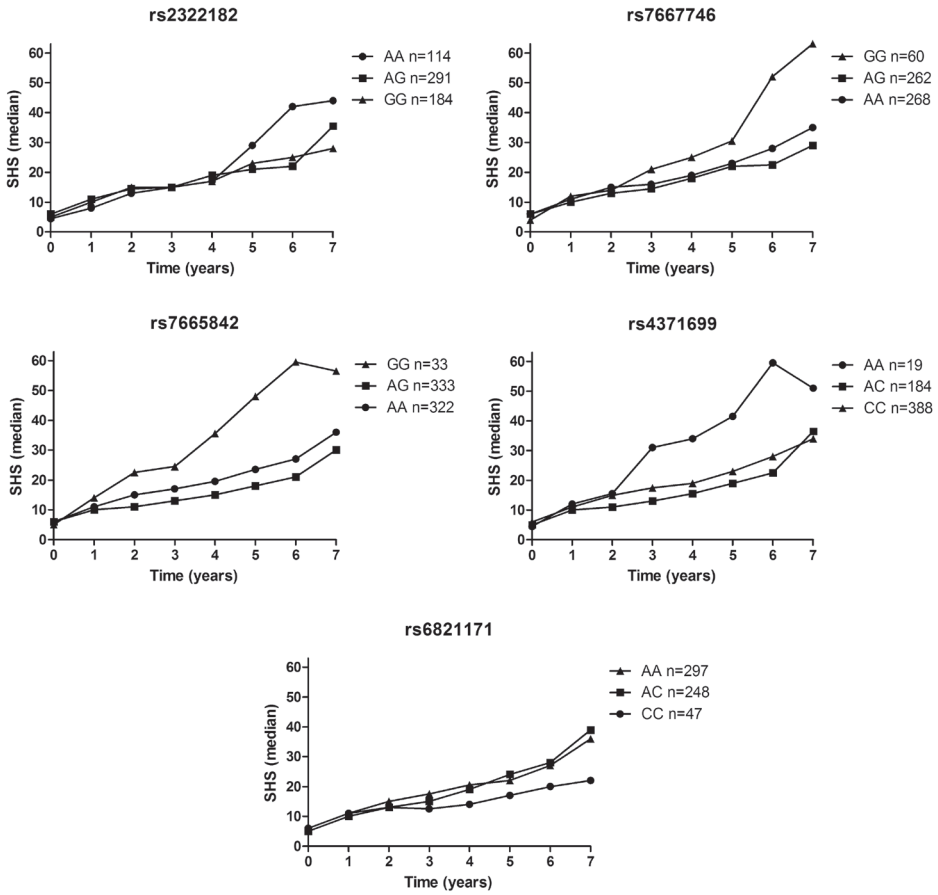


Figure 2 Depicted are the median Sharp-van der Heijde scores during 7-years of follow-up of patients with different genotypes in phase-1 (Leiden-EAC).

Over seven years, patients with twice the minor alleles of rs2322182, rs7667746, rs7665842, rs4371699 and rs6821171 had 1.31 (1.07-1.60, P=0.01), 1.86 (1.43-2.41, P<0.01), 1.88 (1.33-2.65, P<0.01), 1.80 (1.15-2.82, P=0.01) and 0.64 (0.47-0.87, P=0.01) higher rates of joint destruction compared to patient with only one or no minor allele. A beta of 1.09 (rs7667746) per year equals to a 1.86 higher rate of joint destruction over 7 years, which is similar to 86% increase in rate of joint destruction.

data best in all five SNPs. Patients homozygous for the minor allele had respectively 1.04-fold (1.01–1.07, p=0.01), 1.09-fold (1.05–1.13, p<0.01), 1.09-fold (1.04–1.15, p<0.01), 1.09-fold (1.02–1.16, p=0.01) and 0.94-fold (0.90–0.98, p=0.01) higher rates of joint destruction per year as compared to the other patients (table 2). A β of 1.09 per year is equal to a 1.83 (=1.09^7) higher rate of joint destruction over 7 years. Thus, that is, for rs7667746, the estimated rate of joint destruction in patients carrying both minor alleles was 9% higher per year than that of the other patients; over a period of 7 years, this is equal to an 86% (=0.0923^7) higher rate of joint destruction. In a sub-analysis, analyses were also adjusted for anti-CCP, yielding comparable effect sizes (data not shown).

Table 2 Significant associations between IL-15 SNPs and rate of joint destruction as obtained in phase-1 in the Leiden-EAC.

SNP	coordinate	Tested model	Allele minor/ major	MAF	β	95% CI		*P*
rs2322182	142705559	ADD	A/G	0.44	1.02	1.01	1.04	0.01
		REC			1.04	1.01	1.07	$5.0*10^{-3}$
rs7667746	142708334	ADD	G/A	0.33	1.03	1.02	1.05	$2.4*10^{-4}$
		REC			1.09	1.05	1.13	$4.0*10^{-6}$
rs7665842	142713345	ADD	G/A	0.40	1.03	1.01	1.05	0.01
		REC			1.09	1.04	1.15	$3.8*10^{-4}$
rs4371699	142720503	ADD	A/C	0.19	1.03	1.00	1.05	0.03
		REC			1.09	1.02	1.16	$9.4*10^{-3}$
rs6821171	142916618	ADD	C/A	0.29	0.99	0.97	1.01	0.33
		REC			0.94	0.90	0.98	$5.0*10^{-3}$

MAF: minor allele frequency. 95%CI: 95% confidence interval
SNP data is based on NCBI build 35, dbSNP b125
The β of the additive test represents the relative increase in joint destruction per year per minor allele compared to no minor alleles. The β of the recessive test represents the relative increase in joint destruction per year for two minor alleles compared to no one or no minor alleles on the normal scale.

Phase 2

The five significant SNPs were assessed in the other three data sets. Patient characteristics are presented in table 1. One SNP, rs7665842, could not be designed by Sequenom. Instead, a good proxy, rs6835391 (r^2=0.92), was typed. To refer to this proxy, we used rs7665842. Possibly, due to insufficient power, none of the five SNPs were statistically significant when analyzed in each of the other three data sets separately or when tested in the three cohorts combined. Nevertheless, for four of the five SNPs, the effect size in the replication data sets went in the same direction as in the initial data set. The five significant SNPs of phase 1 were analyzed in all 1418 patients of all cohorts in an inverse variance weighting meta-

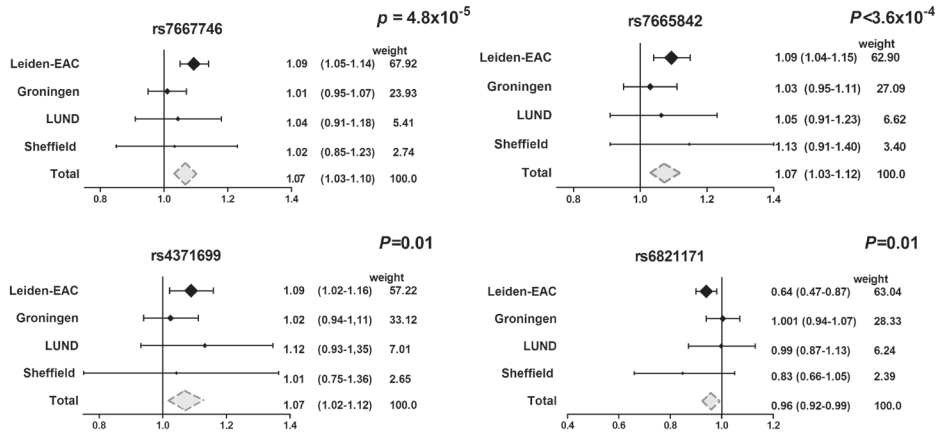


Figure 3 Depicted are the results of the analyses of the individual cohorts and the results of the meta-analyses performed on the four SNPs that were significant in phase-2.
The effect sizes are the estimated relative progression rates per year for the presence of twice the minor allele compared to patients with only one or no minor allele. The p-values in the graphs are uncorrected for multiple testing.
The meta-analyses are based on a fixed effect model, which are applied to genetic studies to test whether there is statistically significant effect; generalisability of the effect is of less importance. As result of this choice, the effect size of the meta-analyses should be considered carefully. Consequently, this methods is less suitable to estimate the effect size overall. Therefore, the estimated effect of the meta-analysis is depicted in gray.
Rs7665842 was typed in the EAC, but could not be designed in the phase-2. Instead rs6835391 (r^2=0.92) was typed in the other three data sets.

analysis in a recessive model. Four SNPs were significant in the meta-analyses: rs7667746 (p<0.001), rs7665842 (p<0.001), rs4371699 (p=0.01) and rs6821171 (p=0.01)(figure 3).

To correct for multiple testing, we calculated the Benjamini– Hochberg false discovery rate q values.[23] This revealed that all four SNPs were significant; p values after multiple testing correction were as follows: rs7667746 (p<0.01), rs7665842 (p<0.01), rs4371699 (p=0.02) and rs6821171 (p=0.03).

Haplotype analysis

In all data sets, the three SNPs associated with enhanced destruction (rs7667746, rs7665842 and rs4371699) were in close LD (r^2=0.46–0.83, D'=0.97–1). To obtain further insight into the associations found, we performed a haplotype analysis. In the Leiden-EAC, two haplotypes with a prevalence >0.1 were found: GGA and AAC (frequencies: 0.19 and 0.67). Analysis of homozygosity for haplotypes against absence resulted in the following for GGA and AAC, respectively: β=1.09 (1.02–1.16, p=0.01) and β=0.97 (0.95–0.998,

p=0.032). Haplotype analysis on the whole gene in the Leiden-EAC resulted in two more blocks (figure 1), which were both not associated with joint destruction (data not shown).

In the three additional data sets, the same haplotypes were made with similar frequencies (frequency: GGA 0.18–0.23 and AAC 0.67–0.69). Meta-analysis of the haplotypes of all data sets resulted in β=1.07 (1.02–1.12, p<0.01) for GGA and β=0.99 (0.97–1.01, p=0.16) for AAC. These effect sizes were comparable to the results of the individual SNPs alone. Therefore, haplotype analyses did not result in additional information on the association discovered.

DISCUSSION

The variance in joint destruction between patients is considerable and is thus far scarcely understood. We performed a candidate gene study to investigate the association of genetic variants with joint destruction. *IL15* (4q31) was chosen as candidate gene since there are emerging data that IL-15 plays a role in perpetuation of inflammation and affects osteoclastogenesis. We tested the association of SNPs tagging *IL15* with rate of joint destruction in one data set and tested the significant SNPs subsequently in three other data sets. Finally, an analysis combining the radiological data of all 1418 patients was performed. Four SNPs were observed to associate significantly with progression of joint destruction. One SNP, rs6821171, had a protective effect. Three SNPs (rs7667746, rs7665842 and rs4371699) that were physically closely related associated with a deteriorative effect on joint destruction. Analysis of these three SNPs together in a haplotype analysis did not further elucidate the discovered associations of the single SNPs.

The aim was to study the evolution of joint destruction in the most unbiased manner. The present study uniquely combines four data sets of patients who started treatment in a time when treatment was not as aggressive as nowadays. Since some of the data sets covered a period where different treatment regimens were used, analyses were adjusted for treatment if relevant.

Replication data sets are ideally larger than the initial data set, since effect sizes are generally smaller at a replication stage. However, relatively few large prospective data sets exist with both x-rays and DNA available in conventionally treated patients. For each data set, the number of patients and the number x-rays were lower than the cohort used in phase 1. The data sets available for phase 2 were possibly underpowered to individually replicate findings. In the absence of independent replication, there is an increased risk of a false-positive finding. To minimise this risk, we summarised data in a meta-analysis to test whether the association still holds. An inverse variance weighting meta-analysis yielded significant results for all four SNPs. Importantly, the effects of the SNPs went into the same direction in each data set, which supports the validity of the results.

Different methods to score joint destruction were applied: Larsen and SHS. Although these methods differ in methodology, sensitivity and ranges of scores, both methods are linearly correlated with each other.[21,25] Therefore, as long as the effects of genetics are tested with the same method within each cohort, the cohort with the different scoring can be combined in one weighted meta-analysis. Nonetheless, the absolute scores provided by both methods are difficult to compare. In the present study, the relative increase in progression rate was evaluated; this relative measure has no units and can therefore be compared.

IL-15 plays a role in T cell proliferation and attraction and has a structural homology with IL-2.[26] IL-15 has pleiotropic and physiological activities in both the innate and acquired immune responses. IL-15 induces T cell proliferation, activates NK-cells, costimulates immunoglobulin synthesis by B cells and activates monocytes.[27] IL-15 is increased in synovial fluid as well as in bone marrow. It is likely to be involved in the perpetuation of inflammation, which consequently may drive progression of joint destruction in RA.

The present data support the notion that genetic variants in *IL15* are involved in the severity of joint destruction. Subsequent studies should elucidate whether the presence of these variants results in difference in IL15 expression, in IL15 activity and in other aspects of IL15 biology.

In conclusion, with a candidate gene approach evaluating patients of four different cohorts, we found an association between genetic variants in *IL15* and rate of joint destruction in RA.

REFERENCE LIST

1. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011;50:93–100.
2. van der Helm-van Mil AH, Kern M, Gregersen PK, et al. Variation in radiologic joint destruction in rheumatoid arthritis differs between monozygotic and dizygotic twins and pairs of unrelated patients. *Arthritis Rheum* 2006;54:2028–30.
3. Asquith DL, McInnes IB. Emerging cytokine targets in rheumatoid arthritis. *Curr Opin Rheumatol* 2007;19:246–51.
4. Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. *J Exp Med* 1995;181:1255–9.
5. McInnes IB, al-Mughales J, Field M, et al. The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat Med* 1996;2:175–82.
6. Kuca-Warnawin E, Burakowski T, Kurowska W, et al. Elevated number of recently activated T cells in bone marrow of patients with rheumatoid arthritis: a role for interleukin 15? *Ann Rheum Dis* 2011;70:227–33.
7. McInnes IB, Liew FY. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. *Immunol Today* 1998;19:75–9.
8. Ferrari-Lacraz S, Zanelli E, Neuberg M, et al. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. *J Immunol* 2004;173:5818–26.
9. Djaafar S, Pierroz DD, Chicheportiche R, et al. Inhibition of T cell-dependent and RANKL-dependent osteoclastogenic processes associated with high levels of bone mass in interleukin-15 receptor-deficient mice. *Arthritis Rheum* 2010;62:3300–10.
10. Petrovic-Rackov L, Pejnovic N. Clinical significance of IL-18, IL-15, IL-12 and TNF- alpha measurement in rheumatoid arthritis. *Clin Rheumatol* 2006;25:448–52.
11. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000;27:261–3.
12. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. *Arthritis Res Ther* 2006;8:R128.
13. Rau R, Herborn G. A modified version of Larsen's scoring method to assess radiologic changes in rheumatoid arthritis. *J Rheumatol* 1995;22:1976–82.
14. Fex E, Jonsson K, Johnson U, et al. Development of radiographic damage during the first 5–6 yr of rheumatoid arthritis. A prospective follow-up study of a Swedish cohort. *Br J Rheumatol* 1996;35:1106–15.
15. Lindqvist E, Jonsson K, Saxne T, et al. Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. *Ann Rheum Dis* 2003;62:611–6.
16. Larsen A, Dale K. Standardized radiological evaluation of rheumatoid arthritis in therapeutic trials. In: Dumonde DC, Jasani MK, eds. The recognition of antirheumatic drugs. *Lancaster: MTP Press,* 1978:285–92.
17. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–5.
18. https://www.wtccc.org.uk
19. Strand V, Landéwé R, van der Heijde D. Using estimated yearly progression rates to compare radiographic data across recent randomised controlled trials in rheumatoid arthritis. *Ann Rheum Dis* 2002;61:ii64–6.
20. van der Helm-van Mil AH, Knevel R, van der Heijde D, et al. How to avoid phenotypic misclassification in using joint destruction as an outcome measure for rheumatoid arthritis? *Rheumatology (Oxford)* 2010;49:1429–35.
21. Lassere M. Pooled metaanalysis of radiographic progression: comparison of Sharp and Larsen methods. *J Rheumatol* 2000;27:269–75.

22. Lebrec JJ, Stijnen T, van Houwelingen HC. Dealing with heterogeneity between cohorts in genomewide SNP association studies. *Stat Appl Genet Mol Biol* 2010;9:Article 8.
23. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;57:289–300.
24. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225–9.
25. Pincus T, Larsen A, Brooks RH, et al. Comparison of 3 quantitative measures of hand radiographs in patients with rheumatoid arthritis: Steinbrocker stage, Kaye modified Sharp score, and Larsen score. *J Rheumatol* 1997;24:2106–12.
26. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu Rev Immunol* 1999;17:19–49.
27. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. *Blood* 2001;97:14–32.

6

Association of Genetic Variants in the *IL4* and *IL4R* Genes With the Severity of Joint Damage in Rheumatoid Arthritis: A Study in Seven Cohorts

A. Krabben, A. G. Wilson, D. P. C. de Rooy, A. Zhernakova, E. Brouwer, E. Lindqvist, T. Saxne, G. Stoeken, J. A. B. van Nies, R. Knevel, T. W. J. Huizinga, R. E. M. Toes, P. K. Gregersen, A. H. M. van der Helm-van Mil

Arthritis & Rheumatology, 2013 December;65(12):3051–3057.

ABSTRACT

Objective

The progression of joint destruction in rheumatoid arthritis (RA) is determined by genetic factors. Changes in *IL4* and *IL4R* genes have been associated with RA severity, but this finding has not been replicated. This study was undertaken to investigate the association between *IL4*- and *IL4R*-tagging single-nucleotide polymorphisms (SNPs) and the progression rate of joint damage in RA in a multi-cohort candidate gene study.

Methods

IL4- and *IL4R*-tagging SNPs (n = 8 and 39, respectively) were genotyped in 600 RA patients for whom 2,846 sets of radiographs of the hands and feet were obtained during 7 years of follow-up. Subsequently, SNPs significantly associated with the progression of joint damage were genotyped and studied in relation to 3,415 radiographs of 1,953 RA patients; these included data sets from Groningen (The Netherlands), Lund (Sweden), Sheffield (UK), the North American Rheumatoid Arthritis Consortium (US), Wichita (US), and the National Data Bank (US). The relative increase in progression rate per year in the presence of a genotype was determined in each cohort. An inverse variance weighting meta-analysis was performed on the 6 data sets that together formed the replication phase.

Results

In the discovery phase, none of the *IL4* SNPs and 7 of the *IL4R* SNPs were significantly associated with the joint damage progression rate. In the replication phase, 2 SNPs in the *IL4R* gene were significantly associated with the joint damage progression rate (rs1805011 [P = 0.02] and rs1119132 [P = 0.001]).

Conclusion

Genetic variants in *IL4R* were identified, and their association with the progression rate of joint damage in RA was independently replicated.

INTRODUCTION

In the last decade it has been recognized that rheumatoid arthritis (RA) needs to be diagnosed early and treated promptly with disease-modifying anti-rheumatic drugs (DMARDs) in order to successfully interfere with the disease process and the progression to joint damage and disability. The progression of joint destruction is highly variable; only a minority of patients develop rapidly progressive disease. To achieve individualized treatment, the severity of the disease outcome needs to be estimated adequately. Ideally, the biologic processes underlying the interindividual differences should be understood. Clinical and serologic risk factors explain only about one-third of the total variance in joint destruction.[1] Genetic variants are estimated to make a major contribution, consisting of 50–60% of the total phenotypic variation.[2] Further studies of individual risk factors are needed to increase understanding of the processes underlying the progression of joint destruction in RA.

We performed a candidate gene study of the association of *IL4* and *IL4R* with the rate of joint destruction in RA. It has been hypothesized that there is an imbalance between Th1 cells and Th2 cells in RA, with different levels of tumor necrosis factor, interleukin-1 (IL-1), IL-6, IL-4, and IL-13.[3] IL-4 mainly promotes differentiation of T cells toward Th2 cells.[4] The role of IL-4 is underscored by observations that the concentration of IL-4 is increased in early arthritis but decreased or absent in synovial fluid from patients with established RA.[4,5] Furthermore, in fibroblast-like synoviocytes, IL-4 was shown to suppress RANKL expression and increase osteoprotegerin expression, and IL-4–knockout mice are characterized by extensive joint destruction,[6] suggesting that IL-4 not only has anti-inflammatory effects, but also has antiosteoclastogenic effects. The effect of IL-4 is mainly mediated by the IL-4 receptor α chain (IL-4Rα).

Several genetic studies of the association of susceptibility to, and the severity of, RA with *IL4* and *IL4R* genes have already been performed. The *IL4* variable-number tandem repeat (VNTR) in the third intron was reported to be associated with lower radiographic damage.[7] Prots et al reported an association between *IL4R* I50V (rs1805010) and the presence of bone erosions in patients with a disease duration of >2 years.[8] Marinou et al, however, found no association of the I50V variant with joint destruction.[9] Furthermore, the *IL4R* Q551R variant (rs1801275) has been studied and found not to be associated with susceptibility to or the severity of RA, but to be associated with the presence of RA nodules.[7-10]

Because of the proposed role of *IL4* and its receptor genes and the observation that the progression of joint damage is in part heritable, we performed a candidate gene study. The genetic variants tagging *IL4* and *IL4R* were determined in 600 patients for whom 2,846 radiographs were available. Furthermore, 3,415 radiographs of 1,953 patients in 6 additional cohorts were subsequently studied for replication.

PATIENTS AND METHODS

Study population

Seven data sets consisting of adult patients diagnosed as having RA according to the American College of Rheumatology 1987 criteria[11] were studied (Table 1). Informed consent was obtained from all patients, and approval was obtained from the local ethics committees.

Discovery phase cohort

Six hundred patients with early RA who were included in the Leiden Early Arthritis Clinic (EAC) from 1993 to 2006 were studied. Radiographs of the hands and feet were obtained at baseline and at yearly follow-up visits for 7 years.[1] A total of 2,846 sets of radiographs of the hands and feet were available. All radiographs were scored by one experienced reader using the modified Sharp/van der Heijde scoring method (SHS).[12] The intra class correlation coefficient (ICC) was 0.91. The treatment received by these patients differed according to 3 treatment periods, as previously described.[1]

Replication phase cohorts

Six data sets were studied (Table 1).[1,13-16] These included 280 patients with 872 radiographs from Groningen, The Netherlands,[1] 391 patients with 391 radiographs from Sheffield, UK,[1,15] 147 patients with 781 radiographs from Lund, Sweden,[1,13] 385 patients with 385 radiographs from the North American Rheumatoid Arthritis Consortium (NARAC) (14), 101 patients with 337 radiographs from Wichita, and 649 patients with 649 radiographs from the National Data Bank (NDB; US).[16]

Single-nucleotide polymorphism (SNP) selection

IL4 and *IL4R* were captured by haploblocks, using the Haploview algorithm, covering the whole gene and 3 kb upstream and downstream of the coding region. Pairwise tagging SNPs were selected from the Utah residents with ancestry from northern and western Europe (CEPH/CEU) HapMap data set (phase II, release 23a/March 2008) using Haploview software (minor allele frequency [MAF] >0.05, pairwise r^2 >0.8). There were no known amino acid–changing SNPs in *IL4* with an MAF of 2:5%. In total, 8 SNPs in *IL4* were tagged with Haploview, without forcing any SNPs.

In *IL4R*, there were 7 known amino acid–changing SNPs. Forty-three SNPs tagged the *IL4R* gene region, with forcing these 7 SNPs. Four SNPs located on *IL4R* failed typing and, therefore, 39 *IL4R* SNPs were successfully tested. The final SNP selection and linkage disequilibrium information are available from the corresponding author upon request.

Table 1 Characteristics of the different cohorts studied

Cohort	No. of RA-patients	No. of X-ray sets	Year of diagnosis	Follow-up, years†	Mean disease duration ±sd, years†	Method of scoring	ICC	ACPA+, n (%)	Age, mean ±sd	Female gender, n (%)
Discovery phase										
Leiden-EAC	600	2,846	1993-2006	7		SHS	0.91	324 (55)	56.3±15.7	419 (69)
Replication phase										
Groningen	280	872	1945-2001	14		SHS	>0.90 /0.96*	163 (80)	49.3±12.6	197 (70)
Lund	147	781	1985-1990	5		Larsen	0.94*	114 (80)	50.7±11.5	98 (67)
Sheffield	391	391	1999-2006		15.4±10.8	Larsen	0.83#	299 (79)	46.0±13.4	285 (73)
NARAC	385	385	1953-2002		13.8±10.5	SHS	0.99	385 (100)	40.8±11.9	281 (73)
Wichita	101	337	1963-1999	15		SHS	0.99	97 (97)	49.0±11.7	70 (69)
NDB	649	649	1972-1999		11.7±6.4	SHS	0.99	523 (81)	47.7±12.7	506 (78)
Total	1,953	3,415								

In Leiden, Groningen, Lund and Sheffield hands and feet X-rays were taken. In the NARAC, Wichita and NDB cohorts hand X-rays were taken.

†For the studies with cross-sectional data (one radiograph in time) the mean disease duration (±sd) at time of the radiograph was reported. The studies with longitudinal data (more than one radiograph in time) the maximum follow-up duration was reported.

SHS Sharp van der Heijde score

ACPA+ anti-citrullinated peptide antibody positivity

All ICC scores represent the correlation coefficient within the reader, accept for the Sheffield cohort#, where a weighted kappa score was provided.

*Furthermore the radiographs in Groningen and Lund were scored by one of two readers. In the Groningen cohort the within reader ICC was >0.90 and the between reader ICC was 0.96. In Lund the between reader ICC was 0.94.

More information on the cohorts is provided in the Supplementary Methods and in references (1;11-14).

SNP genotyping

In all cohorts, DNA was extracted from whole blood using standard methods. In the discovery cohort, genotyping was performed using multiplex SNP arrays designed using an Illumina GoldenGate platform, according to the protocols recommended by the manufacturer. In the cohorts from Groningen, Lund, and Sheffield, SNPs were typed by multiplex SNP arrays designed with a Sequenom iPlex system, according to the protocols recommended by the manufacturer. In the NARAC, genome-wide SNP typing was performed using Infinium HumanHap550, version 1.0 (Illumina). In the Wichita and NDB cohorts, SNPs were typed with an Immunochip, Illumina Infinium High-Density array (Illumina iScan Platform), which was recently designed to densely genotype immune-mediated disease loci identified by genome-wide association studies of common variants using data. The SNPs identified in the discovery phase were retrieved from the genetic databases of the NARAC, Wichita, and NDB cohorts. Four of the SNPs identified were not available in the genetic databases for the Wichita and NDB cohorts.

Statistical analysis

In all data sets, 1 was added to all radiologic scores and then the scores were log-transformed to obtain a normal distribution. Two phases were carried out. First, an explorative analysis was performed in the Leiden EAC cohort, testing the tagged SNPs both additively and recessively. A multivariate normal regression model for longitudinal data was used with repeated radiologic score as a response variable. This model makes use of repeated radiologic measurements by taking advantage of within-patient correlation, yielding more precise estimates of the progression rates and therefore increasing the power to detect differences. This model uses a covariance matrix, allowing the inclusion of patients who had missing radiographs at some point during follow-up. The model fit was tested by residual analyses.[1,17] Adjustment variables were entered based on their single variable association with joint destruction. Adjustments were made for age, sex, and the treatment periods described previously.[1]

In the second, replication, phase only the model (recessive or additive) that fit best in the discovery phase was tested. Each of the individual replication cohorts had fewer radiographs and hence less power than the discovery cohort. Because of differences in study designs, the data from the separate replication cohorts could not be combined into one analysis directly. Therefore, we decided to test the SNPs in each data set separately, taking advantage of the specific data set characteristics, and to subsequently perform a meta-analysis. Since the beta values obtained from the analyses of the different cohorts all reflected the relative increase in radiologic progression rate per year in patients with a certain genotype compared to patients with the common genotype, the estimates could be pooled in a meta-analysis.

In the Groningen, Lund, and Wichita cohorts, multiple radiographs per individual were available, and a multivariate normal regression analysis was used, which was similar to the analysis applied in the Leiden EAC cohort. Adjustment variables were entered based on their single variable association with joint destruction. The Groningen data set was adjusted for age and inclusion before or after 1990, as a proxy for DMARD therapy. The analysis of the Lund cohort was adjusted for age only, since sex and treatment were not associated with joint destruction in this data set. The analysis of the Wichita cohort was adjusted for age and sex.

In the Sheffield, NARAC, and NDB data sets, radiographs were obtained from each patient at one time point. To derive estimates of the radiologic progression, the estimated yearly progression rate was calculated by dividing the total SHS by the number of years of disease duration at the time the radiograph was obtained. Also in this analysis, 1 was added to the estimated yearly progression rate data before log-transformation. Subsequently, the SNP association was tested in a linear regression analysis with log-transformed estimated yearly progression rate as the outcome variable. The resulting estimate reflected how many fold the rate of joint destruction increases per year in patients with a certain genotype compared to patients with the common genotype. No adjustments were applied to the analysis of the Sheffield and NARAC data sets, since none of the variables tested were significantly associated with joint destruction. Analyses of the NDB data set were adjusted for age and sex. SPSS version 17.0 was used.

Since all of the beta values obtained reflected the relative increase in radiologic progression rate (a relative measure without units), the beta values and standard errors could be combined in a fixed-effects meta-analysis with inverse variance weighting.[18] The standard errors differed between the data sets because the number of radiographs per patient differed between the cohorts, resulting in more precise estimates of the relative progression rates and smaller standard errors in data sets with serial measurements. The meta-analysis was performed in Stata, version 10.1.

Multiple testing increases the possibility of incorrectly rejecting the null hypothesis to >5%. Therefore, Bonferroni correction for multiple testing was applied in phase 2.

Haplotype analysis

Haplotypes of *IL4R* were studied. Haplotype blocks were defined using the model described by Gabriel et al.[19] Haplotypes were assigned to each individual using Plink version 1.07. Analyses of the haplotypes were performed with methods similar to those used for the analyses of the individual SNPs by additive testing of a haplotype.

RESULTS

Discovery phase

A complete overview of the results of all SNPs studied in the discovery phase is available from the corresponding author upon request. None of the 8 SNPs tagging *IL4* were significantly associated with the progression of joint destruction in the Leiden EAC cohort.

Analysis of the 39 SNPs in the *IL4R* gene region revealed 7 SNPs that were significantly associated with the progression of joint destruction (Table 2). For 4 SNPs, the recessive analysis showed the strongest association (rs4787423 [*P* = 0.03], rs7191188 [*P* < 0.01], rs6498016 [*P* = 0.01], and rs1119132 [*P* = 0.04]), and for 3 SNPs, the additive analysis showed the strongest association (rs1805011 [*P* = 0.01], rs1805015 [*P* = 0.04], and

Table 2 Results of the SNPs in *IL-4R* with a significant association with the radiological progression rate in the discovery phase.

		Discovery phase						Replication phase (meta-analysis)	
SNP	Coordinate	MAF EAC	Tested model	β	95%CI		P	No. cohorts	P
rs4787423	27274835	0.14	ADD	0.99	0.96	1.01	0.35		
			REC	0.90	0.83	0.99	0.03	4	0.81
rs1805011	27281373	0.11	ADD	0.96	0.93	0.99	0.01	4	0.02
			REC	0.93	0.81	1.07	0.34		
rs1805015	27281681	0.16	ADD	0.98	0.95	1.00	0.04	4	0.08
			REC	0.99	0.91	1.07	0.76		
rs1801275	27281901	0.20	ADD	0.97	0.95	0.99	0.01	4	0.21
			REC	0.99	0.93	1.05	0.67		
rs7191188	27296912	0.25	ADD	1.02	1.01	1.04	0.01		
			REC	1.10	1.05	1.15	<0.01	6	0.83
rs6498016	27299289	0.21	ADD	1.01	1.00	1.03	0.16		
			REC	1.08	1.02	1.14	0.01	6	0.88
rs1119132	27310970	0.13	ADD	1.02	1.00	1.04	0.15		
			REC	1.09	1.00	1.18	0.04	6	0.001

The β indicates the fold difference in progression rate in the presence of the risk allele or risk genotype. The β from the additive model indicates the fold difference in progression rate for each additive minor allele present. The β from the recessive model indicates the fold difference in progression rate in patients homozygous for the minor allele versus the other patients. For instance patients carrying one minor allele of rs1805011 had a 0.96-fold higher rate of joint destruction compared to patients without a minor allele. This corresponds with a 24% (0.961^7=0.76) lower rate of joint destruction over 7 years.
In the replication phase, analyses were only performed for the model with the highest significance level in the discovery phase. rs4787423, rs1805011, rs1805015 and rs1801275 were not available in the genetic database of Wichita and NDB. An inverse weighted meta-analysis was performed on 4 or 6 independent cohorts. In the NARAC, Wichita and NDB cohort a proxy SNP for rs1119132 was analyzed; rs1859308 (r^2=0.92). In the NARAC cohort a proxy for rs4787423 was analyzed; rs3024660 (r^2=1).

rs1801275 [$P = 0.01$]) (Table 2). In this exploratory phase, no corrections for multiple testing were made, and these 7 variants were studied in the second phase.

Replication phase

A total of 1,953 patients and 3,415 scored radiographs were evaluated. The SNPs rs7191188, rs6498016, and rs1119132 were studied in all 6 cohorts (Groningen, Lund, Sheffield, NARAC, Wichita, and NDB). For rs1805011, rs4787423, rs1805015, and rs1801275, no genotyping data were available for the Wichita and NDB cohorts; hence, these SNPs were studied in 4 cohorts (Groningen, Lund, Sheffield, and NARAC). In addition, proxy SNPs were analyzed for 2 SNPs in some cohorts. In the NARAC, Wichita, and NDB cohorts, a proxy SNP for rs1119132 was analyzed: rs1859308 ($r^2 = 0.92$). In the NARAC cohort, a proxy SNP for rs4787423 was analyzed: rs3024660 ($r^2 = 1$). Since all replication cohorts had fewer available radiographs than the discovery cohort, the power to find significant differences in each of these cohorts was limited, and data were combined in a meta-analysis. The analyses were performed by testing either a recessive or an additive association, depending on the results obtained in the discovery phase. Of the 7 SNPs studied in this phase, 2 were significantly associated with joint progression. These were rs1805011 ($P = 0.02$) and rs1119132 ($P = 0.001$) (Table 2 and Figure 1). These SNPs are in low linkage disequilibrium ($r^2 = 0.01$). In a conditional analysis including both SNPs, both remained significant (rs1805011 [$P = 0.006$] and rs1119132 [$P = 0.041$]), indicating that their effect is likely to be independent. After Bonferroni correction for multiple testing for 7 SNPs in the replication phase, rs1119132 remained significantly associated with the progression of joint damage ($P_{corrected} = 0.007$).

Findings of haplotype analysis

To attempt to further elucidate the associations found, a haplotype analysis was performed using the data for the *IL4R* gene in the Leiden EAC cohort. This resulted in 9 haplotype blocks with a minor haplotype frequency of >0.01 (results available from the corresponding author upon request). All haplotypes with a prevalence of >0.1 were tested for an association with joint destruction in the EAC cohort. Because of this cutoff, no haplotypes that included rs119132 were evaluated. Two haplotypes of one haplotype block showed a better association than the independent SNPs (AAA and CGG, consisting of the following SNPs: rs1805011, rs1805015, and rs1801275, respectively). These haplotypes were analyzed in the replication phase, and no association with the progression of joint damage was observed.

Finally, rs1119132 and rs1805011 were studied in anti–citrullinated protein antibody (ACPA)–positive and ACPA-negative patients separately. This yielded comparable effect sizes in both subgroups (data not shown).

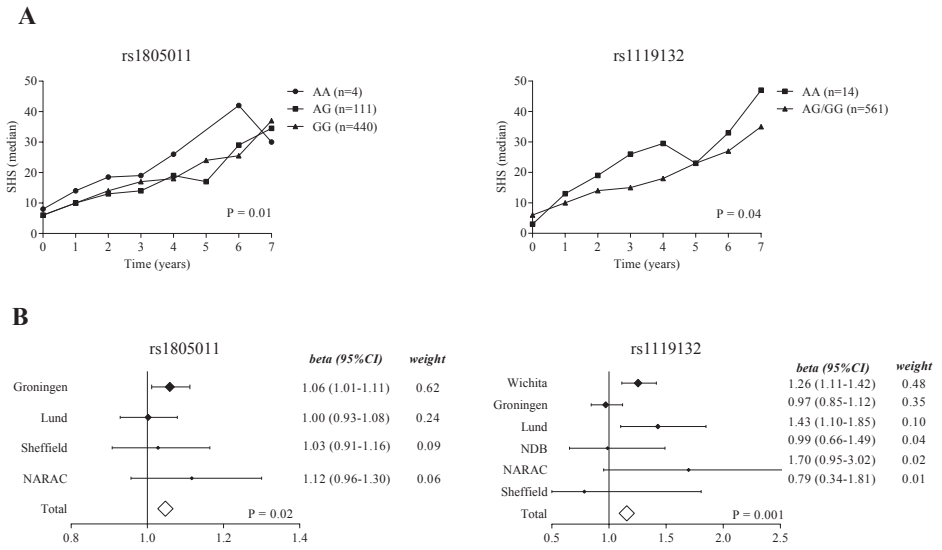


Figure 1 Results of rs1805011 and rs1119132 in the discovery phase (A) and replication phase (B).
A. Presented are the median Sharp van der Heijde scores (SHS) over 7-years of follow-up, per genotype in RA-patients of the EAC. The 'bump' in the line at year five is caused by missing radiographs of part of the patients with rs1119132 genotype AA.
B. Presented is an inverse variance weighted meta-analysis in four cohorts (rs1805011) and six cohorts (rs1119132). In the NARAC, Wichita and NDB cohort a proxy SNP for rs1119132 was analyzed; rs1859308 (r^2=0.92). Genotyping data of rs1805011 were not available for the Wichita and NDB cohort.

DISCUSSION

The severity of RA is reflected by the severity of radiologic joint destruction. It is highly variable between patients, and part of this variance is explained by genetic factors. Several studies of IL-4 at the protein level have suggested that this interleukin is relevant in RA.[3-6] In addition, several genetic studies of *IL4* and *IL4R* and joint damage have been performed,[7-10] though none of the factors identified have been replicated. This prompted us to perform the present multi-cohort candidate gene study. We observed that patients carrying 2 minor alleles of rs1119132 in *IL4R* had more severe joint damage progression. Although a minority of RA patients may have this genetic variant, individual independent replication was found in some of the replication cohorts as well as in the meta-analysis of the 6 replication cohorts.

In addition to the results for rs1119132, another SNP in *IL4R*, rs1805011, showed an association with joint destruction. In a conditional analysis including both SNPs, both remained significantly associated with the progression of joint damage. These SNPs were in low linkage disequilibrium. Since, after applying the conservative Bonferroni correction for

multiple testing only rs1119132 remained significant, we did not draw a definite conclusion regarding rs1805011.

SNP I50V (rs1805010) in *IL4R* was previously found to be associated with joint destruction in RA.[8] Despite studies of the potential functional relevance of this SNP,[8,20] the association of this variant with joint damage was not observed in the study by Marinou et al[9] or in our study. Another coding variant on *IL4R*, Q551R (rs1801275), was not associated with joint destruction in prior studies.[7-10] In the present study, *IL4R* Q551R was significantly associated with joint destruction in the discovery cohort ($P = 0.01$) but not in the replication phase ($P = 0.21$).

Despite previous in vitro studies and mouse studies showing that IL-4 plays a role in suppressing arthritis severity, in the present study no association between SNPs in *IL4* and joint destruction were observed. An association between *IL4* VNTR has been reported previously.[7] This variant was not included in our study.

We used the classic candidate gene approach, including immune response factors that had previously been shown to be involved in RA pathogenesis. This method has a larger a priori chance of finding a true association between SNPs and disease severity. However, this approach may also result in false-positive or false-negative findings. We studied 6 replication cohorts in order to reduce the chance of false-positive findings.

In conclusion, we identified and replicated a genetic variant in *IL4R* predisposing to joint damage progression in RA. Further studies of IL-4R at the protein level are needed to increase insight on the role of this variant in the pathogenesis of RA progression.

REFERENCE LIST

1. Knevel R, Krabben A, Brouwer E, et al. Genetic variants in IL15 associate with progression of joint destruction in rheumatoid arthritis: a multicohort study. *Ann Rheum Dis* 2012;71:1651–7.
2. Knevel R, Grondal G, Huizinga TW, et al. Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. *Ann Rheum Dis* 2012;71:707–9.
3. Van Roon JA, Verhoef CM, van Roy JL, et al. Decrease in peripheral type 1 over type 2 T cell cytokine production in patients with rheumatoid arthritis correlates with an increase in severity of disease. *Ann Rheum Dis* 1997;56:656–60.
4. Miossec P, Naviliat M, Dupuy D'Angeac A, et al. Low levels of interleukin-4 and high levels of transforming growth factor beta in rheumatoid synovitis. *Arthritis Rheum* 1990;33:1180–7.
5. Raza K, Falciani F, Curnow SJ, et al. Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. *Arthritis Res Ther* 2005;7:R784–95.
6. Tunyogi-Csapo M, Kis-Toth K, Radacs M, et al. Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: fibroblast-mediated pathologic bone resorption. *Arthritis Rheum* 2008; 58:2397–408.
7. Genevay S, Di Giovine FS, Perneger TV, et al. Association of interleukin-4 and interleukin-1B gene variants with Larsen score progression in rheumatoid arthritis. *Arthritis Rheum* 2002;47:303–9.
8. Prots I, Skapenko A, Wendler J, et al. Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006;54:1491–500.
9. Marinou I, Till SH, Moore DJ, et al. Lack of association or interactions between the IL-4, IL-4Rex and IL-13 genes, and rheumatoid arthritis. *Arthritis Res Ther* 2008;10:R80.
10. Burgos PI, Causey ZL, Tamhane A, et al. Association of IL4R single-nucleotide polymorphisms with rheumatoid nodules in African Americans with rheumatoid arthritis. *Arthritis Res Ther* 2010;12:R75.
11. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
12. Van der Heijde DM, van Riel PL, Gribnau FW, et al. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet* 1989;1:1036–8.
13. Fex E, Jonsson K, Johnson U, et al. Development of radiographic damage during the first 5-6 yr of rheumatoid arthritis: a prospective follow-up study of a Swedish cohort. *Br J Rheumatol* 1996;35:1106–15.
14. Jawaheer D, Seldin MF, Amos CI, et al, for the North American Rheumatoid Arthritis Consortium. Screening the genome for rheumatoid arthritis susceptibility genes: a replication study and combined analysis of 512 multicase families. *Arthritis Rheum* 2003;48:906–16.
15. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. *Arthritis Res Ther* 2006;8: R128.
16. Wolfe F, Michaud K. The National Data Bank for rheumatic diseases: a multi-registry rheumatic disease data bank. *Rheumatology (Oxford)* 2011;50:16–24.
17. Knevel R, Tsonaka R, le Cessie S, et al. Comparison of methodologies for analysing the progression of joint destruction in rheumatoid arthritis. *Scand J Rheumatol* 2013;42:182–9.
18. Lassere M. Pooled metaanalysis of radiographic progression: comparison of Sharp and Larsen methods. *J Rheumatol* 2000;27: 269–75.
19. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science* 2002;296:2225–9.
20. Wallis SK, Cooney LA, Endres JL, et al. A polymorphism in the interleukin-4 receptor affects the ability of interleukin-4 to regulate Th17 cells: a possible immunoregulatory mechanism for genetic control of the severity of rheumatoid arthritis. *Arthritis Res Ther* 2011;13:R15.

SUPPLEMENTARY METHODS

Brief description of replication cohorts

Groningen 280 RA-patients from the Northern part of The Netherlands, diagnosed between 1945 and 2001 were studied. Over a follow-up duration of at most 14 years the mean number of X-ray sets (hands and feet) per patient was 3.1 (with a maximum of eight X-rays per patient). The total number of sets of X-rays was 872. The X-rays were scored by one of two readers using SHS. ICCs within readers were >0.90 and between readers 0.96. Patients included in the 90's were treated with DMARD therapy in contrast to patients included before the 1990.

Lund 147 early RA-patients from Sweden, recruited from primary care units in the area of Lund during the years 1985-1990 were studied. They were prospectively followed yearly during 5 years. In total 781 sets of X-rays were available and scored according to Larsen by one of two readers. The ICC between the readers was 0.94. In the inclusion period, immediate DMARD therapy was not common and only half of the patients used any DMARD at 5 years follow-up.

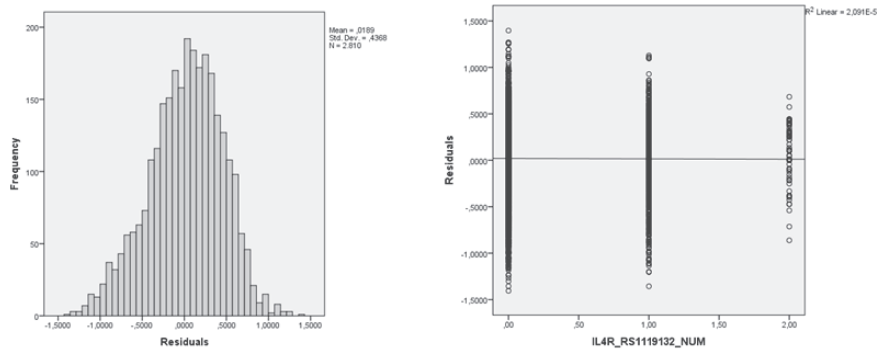
Sheffield 391 RA-patients from the area of Sheffield (UK) recruited between 1999 and 2006 were evaluated. RA-patients were assessed once during their disease course. The mean (±SD) disease duration at assessment was 15±11 years (range 3-65 years). X-rays of hands and feet were scored by one reader using a modification to Larsen's score. The intra-observer variation by a weighted kappa score was 0.83.

NARAC 385 ACPA-positive RA-patients from the North American Rheumatoid Arthritis Consortium who were radiographed between 1953 and 2002, with cross-sectional radiological measurements of hands, were studied. The mean (±sd) disease duration at assessment was 14±11 years. The radiographs were scored according to SHS by a single reader, with an ICC of 0.99.

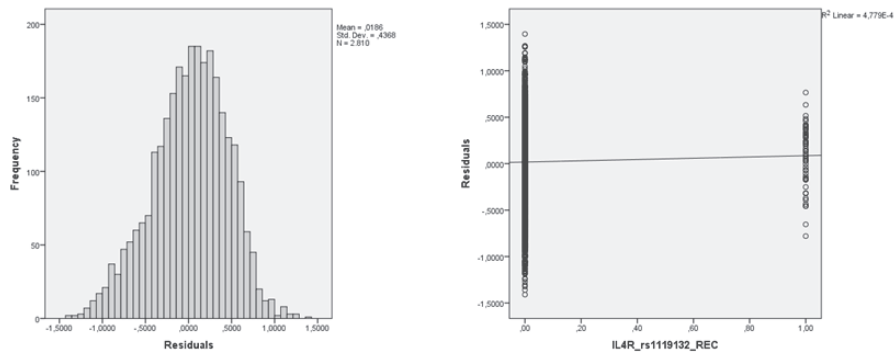
Wichita 101 patients from one practice in Wichita (Kansas, USA), recruited between 1963 and 1999 were studied. Patients were followed for a maximum of 15 years. Radiographic data were obtained when needed for clinical care. In total, 337 sets of hands X-rays were available. All X-rays were scored with SHS by one reader, the within reader ICC was 0.99.

NDB 649 patients included in the National Databank for Rheumatic diseases between 1972 and 1999, a databank that consists of patients with rheumatic diseases from the USA and Canada, were studied. A single time-point X-ray of the hands was available over a follow-up duration of at most 25 years with a mean (±sd) of 12±6 years. All X-rays were scored according to the SHS by the same reader who scored the Wichita X-rays.

Additive model:

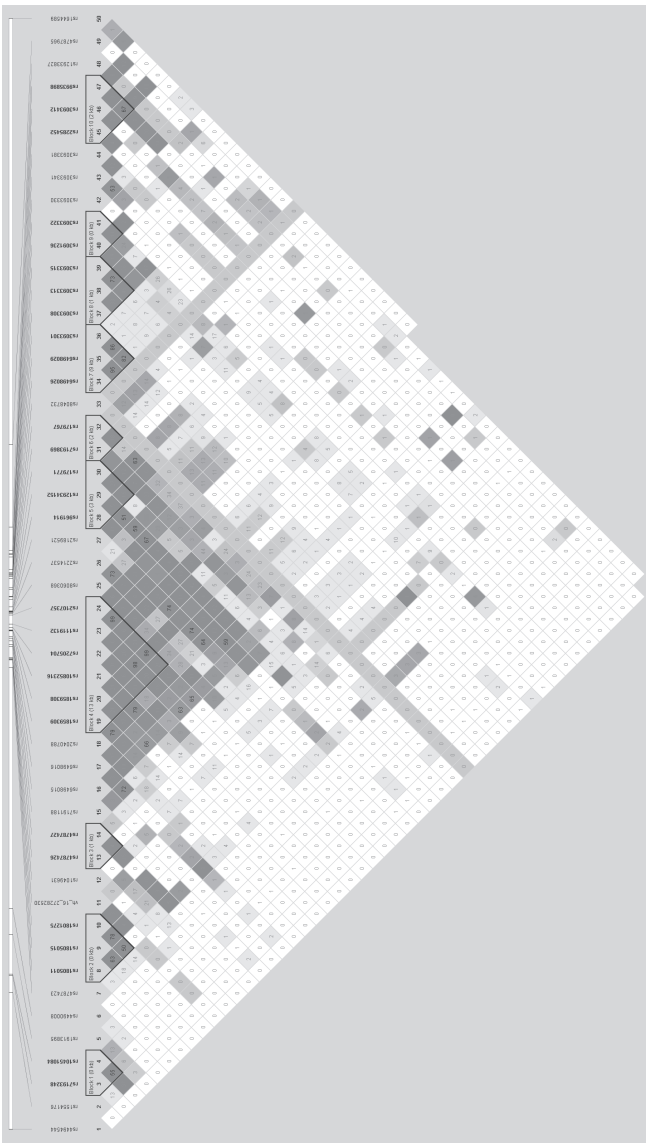


Recessive model:



Supplementary Figure 1 Residual analyses of the model used in the discovery cohort, including all covariates and rs1119132.

These graphs demonstrate the normal distribution of the residuals and that there is no correlation between genotype and residuals, meaning that the model fits the data.



Supplementary Figure 2 LD structure between the 43 SNPs in *IL4-R* in the Leiden-EAC
Presented are the nine haploblocks composed of 43 SNPs in the *IL-4R* gene and with a minor haplotype frequency (MHF) > 0.01. All haplotypes with a prevalence >0.1 were tested with joint destruction. Two haplotypes (AAA and CGG; rs1805011, rs1805015 and rs1801275, respectively) of one haploblock (depicted as block 5) showed a better association with joint destruction then the independent SNPs. Analysis of the additional presence of the haplotype in the discovery cohort, resulted in the following results for AAA β=1.02, 95%CI=1.00-1.05, p=0.02 and for CGG β=0.97, 95%CI=0.94-1.00 p=0.02. AAA and CGG had a frequency of 0.80 and 0.11 in the EAC (discovery cohort).
The frequencies of the AAA and CGG haplotypes were similar in the four additional datasets (frequency 0.75-0.79 and 0.10-0.15). Meta-analysis of the haplotypes of all datasets resulted in not significant results. For additive testing of the haplotypes it resulted in p=0.31 for AAA and p=0.64 for CGG.

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase

gene	rs number	coordinate	model	p
IL-4R	rs3024530	27258188	ADD	n.s.
			REC	n.s.
	rs4787426	27292232	ADD	n.s.
			REC	n.s.
	rs1805011	27281373	ADD	0.006
			REC	0.335
	rs11074852	27208097	ADD	n.s.
			REC	n.s.
	rs4547335	27204191	ADD	n.s.
			REC	n.s.
	rs1805010	27263704	ADD	n.s.
			REC	n.s.
	rs7205704	27308394	ADD	n.s.
			REC	n.s.
	rs10852316	27306056	ADD	n.s.
			REC	n.s.
	rs1029489	27283718	ADD	n.s.
			REC	n.s.
	rs4787423	27274835	ADD	0.354
			REC	0.028
	rs1801275	27281901	ADD	0.011
			REC	0.672
	rs1805015	27281681	ADD	0.037
			REC	0.764
	rs4787956	27285750	ADD	n.s.
			REC	n.s.
	rs3024560	27264168	ADD	n.s.
			REC	n.s.
	rs2040788	27300443	ADD	n.s.
			REC	n.s.
	rs9944340	27301092	ADD	n.s.
			REC	n.s.
	rs1805012	27281465	ADD	n.s.
			REC	n.s.
	rs1119132	27310970	ADD	0.147
			REC	0.038
	rs7191188	27296912	ADD	0.008
			REC	<0.001

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase (continued)

gene	rs number	coordinate	model	p
	rs6498016	27299289	ADD	0.164
			REC	0.006
	rs12102586	27285554	ADD	n.s.
			REC	n.s.
	rs11648218	27317583	ADD	n.s.
			REC	n.s.
	rs16976728	27289213	ADD	n.s.
			REC	n.s.
	rs8044444	27236044	ADD	n.s.
			REC	n.s.
	rs2234895	27265428	ADD	n.s.
			REC	n.s.
	rs8050048	27208885	ADD	n.s.
			REC	n.s.
	rs8052962	27234343	ADD	n.s.
			REC	n.s.
	rs4787427	27293895	ADD	n.s.
			REC	n.s.
	rs2074570	27282658	ADD	n.s.
			REC	n.s.
	rs4787948	27248560	ADD	n.s.
			REC	n.s.
	rs3024622	27272954	ADD	n.s.
			REC	n.s.
	rs7204874	27210788	ADD	n.s.
			REC	n.s.
	rs1049631	27283043	ADD	n.s.
			REC	n.s.
	rs2239347	27266522	ADD	n.s.
			REC	n.s.
	rs6498015	27299125	ADD	n.s.
			REC	n.s.
	rs3024613	27271754	ADD	n.s.
			REC	n.s.
	rs4238954	27221612	ADD	n.s.
			REC	n.s.
	rs4787947	27226266	ADD	n.s.
			REC	n.s.

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase (continued)

gene	rs number	coordinate	model	p
	rs8056488	27210230	ADD	n.s.
			REC	n.s.
IL-4	rs2243248	132036543	ADD	n.s.
			REC	n.s.
	rs6864565	132075870	ADD	n.s.
			REC	n.s.
	rs2243263	132041198	ADD	n.s.
			REC	n.s.
	rs17691077	132071250	ADD	n.s.
			REC	n.s.
	rs3756752	132101772	ADD	n.s.
			REC	n.s.
	rs6883504	132061021	ADD	n.s.
			REC	n.s.
	rs17623617	132060397	ADD	n.s.
			REC	n.s.
	rs1080001	132077015	ADD	n.s.
			REC	n.s.

*p value >0.05 was assumed not significant (n.s.). ADD = additive model and REC= recessive model.

7

A Genetic Variant in *Granzyme-B* Is Associated With Progression of Joint Destruction in Rheumatoid Arthritis

R. Knevel, A. Krabben, A. G. Wilson, E. Brouwer, M. K. Leijsma,
E. Lindqvist, D. P. C. de Rooy, N. A. Daha,
M. P. M. van der Linden, S. Tsonaka, A. Zhernakova,
H. J. Westra, L. Franke, J. J. Houwing-Duistermaat,
R. E. M. Toes, T. W. J. Huizinga, T. Saxne,
A. H. M. van der Helm-van Mil

Arthritis & Rheumatology, 2013 March;65(3):582–589.

ABSTRACT

Objective

Genetic factors account for an estimated 45–58% of the variance in joint destruction in rheumatoid arthritis (RA). The serine proteinase granzyme B induces target cell apoptosis, and several in vitro studies suggest that granzyme B is involved in apoptosis of chondrocytes. Serum levels of granzyme B are increased in RA and are also associated with radiographic erosions. The aim of this study was to investigate *GZMB* as a candidate gene accounting for the severity of joint destruction in RA.

Methods

A total of 1,418 patients with 4,885 radiograph sets of the hands and feet from 4 independent cohorts were studied. First, explorative analyses were performed in 600 RA patients in the Leiden Early Arthritis Clinic cohort. Fifteen single-nucleotide poly-morphisms (SNPs) tagging *GZMB* were tested. Significantly associated SNPs were genotyped in data sets representing patients from the Groningen, Sheffield, and Lund cohorts. In each data set, the relative increase in the annual rate of progression in the presence of a genotype was assessed. Data were summarized in a meta-analysis. The association of *GZMB* with the RNA expression level of the *GZMB* genomic region was tested by mapping expression quantitative trait loci (QTLs) on 1,469 whole blood samples.

Results

SNP rs8192916 was significantly associated with the rate of joint destruction in the first cohort and in the meta-analysis of all data sets. Patients homozygous for the minor allele of rs8192916 had a higher rate of joint destruction per year compared with other patients (P 7.8 x 10^{-4}). Expression QTL of *GZMB* identified higher expression in the presence of the minor allele of rs8192916 (P 2.27 x 10^{-5}).

Conclusion

SNP rs8192916 located in *GZMB* is associated with the progression of joint destruction in RA as well as with RNA expression in whole blood.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder that affects 0.5–1% of the population and is associated with significant morbidity, disability, and cost to society. Radiographic joint destruction reflects the cumulative burden of inflammation and is considered to be an objective measure of the severity of RA[1]. The degree of joint destruction varies significantly between patients. The processes behind this difference are incompletely understood. Inflammatory markers and auto-antibodies are potent risk factors for joint destruction but explain ~30% of the variance in joint destruction[2]. Results of a twin study suggested that genetic factors influence the severity of joint destruction in RA, and a recent study in the Icelandic RA population estimated the heritability rate of joint destruction to be ~45–58%.[3,4] Hence, to increase understanding of the processes mediating joint destruction, it would be beneficial to study genetic variants that could predispose to a severe outcome of RA.

Here, we describe a candidate gene study of the association of *GZMB* with the rate of joint destruction. Granzyme B is a serine protease found in the lytic granules of natural killer (NK) cells and cytotoxic T lymphocytes. When granzyme B is secreted into the interspace between the NK cell and the target cell, it triggers cell death by apoptosis.[5,6] In vitro studies have shown that granzyme B has enzymatic activity for the cleavage of aggrecan proteoglycans from cultured cartilage matrix and whole cartilage explants;[7,8] loss of cartilage proteoglycans is an early event in the course of destructive arthritis.[9] In addition, studies of cartilaginous tissue demonstrated the presence of many granzyme B–positive cells among the chondrocytes in the pannus lesion.[8] Furthermore, the levels of granzyme B were shown to be increased in the synovial fluid of patients with RA compared with that of healthy control subjects,[10] and increased serum levels were associated with early development of radiographic erosions.[11] Finally, a genetic variant (rs854350) in *GZMB* is reported to associate with susceptibility to RA.[12]

These data led us to hypothesize that genetic variants in *GZMB* are associated with the severity of joint destruction in RA. We tested this hypothesis using 4 data sets comprising data for European RA patients for whom longitudinal radiography data on joint destruction were available. All data sets included patients in whom the diagnosis was made during a period when treatment strategies were less aggressive and less controlled than they are currently. These conservative treatment strategies made the data sets suitable for the present study, because the natural disease course was less inhibited. To further investigate the findings, the single-nucleotide polymorphisms (SNPs) associated with progression of joint destruction were also tested for their association with RNA expression.

PATIENTS AND METHODS

Study population

Four data sets comprising data for adult European patients with RA were studied. RA was defined according to the 1987 American College of Rheumatology criteria[13] in all data sets except the Lund data set, for which the 1958 criteria for RA[14] were used. Radiographs of both the hands and feet were available for all patients (Table 1). All patients provided informed consent, and approval was obtained from the local ethics committee for each study.

Table 1: Characteristics for each dataset.

Cohort	Leiden-EAC (n=600)	Groningen (n=275)	Sheffield (n=396)	Lund (n=147)
Year of diagnosis	1993-2006	1945-2001	1938-2003	1985-1990
Follow-up years*	7 years	14 years	Not applicable*	5 years
Total no. of X-ray sets	2,846	862	396	781
Method of scoring	SHS	SHS	Larsen	Larsen
Female n (%)	412 (69)	194 (71)	290 (73)	98 (67)
Age at diagnosis, mean ± SD	56 ± 16	49 ± 13	46 ± 13	51 ± 12
Anti-CCP+ n (%)	323 (55)	160 (80)	302 (79)	114 (80)

SHS= Sharp-van der Heijde score

*Data of Leiden-EAC, Groningen and Lund were from baseline onwards during respectively 7, 14 and 5 years of follow-up. The data of Sheffield were collected once during the disease period, the mean disease duration was 15 years (range 3-65 years).

Leiden Early Arthritis Clinic (EAC) cohort

This data set represented 600 patients with early RA from the western part of The Netherlands, who were included in the Leiden EAC between 1993 and 2006.[2] Patients were included at the time of diagnosis and were followed up annually. Radiographs were obtained at baseline and at yearly follow-up visits for 7 years. A total of 2,846 sets of radiographs of the hands and feet were available. All radiographs were chronologically scored by one experienced reader who was unaware of the genetic or clinical data, using the Sharp/van der Heijde (SHS) scoring method for the hands and feet.[15] A total of 499 randomly selected radiographs were scored twice. The within-reader intra class correlation coefficient (ICC) was 0.91. Treatment of the patients could be divided into 3 time periods. Patients included in 1993–1995 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996–1998 were initially treated with chloroquine or sulfasalazine, and patients included after 1999 were promptly treated with methotrexate or sulfasalazine.

Groningen cohort

The second set of data represented 275 RA patients from the northern part of The Netherlands, in whom RA was diagnosed between 1945 and 2001. The duration of follow-up after diagnosis was limited to 14 years. The mean number of radiograph sets (hands and feet) per patient was 3.1 (maximum of 8 radiographs per patient). The total number of radiograph sets was 862. The radiographs were scored chronologically by 1 of 2 readers, using the SHS method. The ICC within readers was >0.90 and between readers was 0.96. In this data set, patients in whom RA was diagnosed before 1990 had, on average, a more progressive course of joint destruction compared with patients in whom RA was diagnosed after 1990. This difference in joint destruction progression is consistent with the approach of initiating disease-modifying anti-rheumatic drug (DMARD) treatment early, which was introduced in the 1990s.

Sheffield cohort

The third data set represented 396 RA patients from the Sheffield, UK area. RA patients for whom radiographs were available were recruited from the Rheumatology Department of the Royal Hallamshire Hospital in Sheffield between 1999 and 2006.[16] RA patients were assessed once during their disease course. The mean ± SD disease duration at the time of the assessment was 15 ± 11 years (range 3–65 years). Radiographs of the hands and feet were scored by one reader using a modification of the Larsen score.[17] Ten percent of the radiographs were scored twice to quantify intra-observer variation, using a weighted kappa value of 0.83.[16]

Lund cohort

The fourth data set represented 183 Swedish patients with early RA who were prospectively followed up yearly over 5 years; radiographs and DNA were available for 147 of these patients.[18,19] Patients were recruited from primary care units in the Lund area from 1985 to 1989. Radiographs of the hands and feet were obtained at the start of the study and then annually for 5 years, resulting in a total of 781 sets of radiographs. Radiographs were scored chronologically by one of two readers, according to the Larsen method.[20] The ICC between readers, as determined based on 105 radiographs, was 0.94. During the inclusion period, immediate DMARD therapy was not common, and only half of the patients were receiving any DMARD at the 5-year follow-up. The DMARDs used most commonly were chloroquine, D-penicillamine, sodium aurothiomalate, and auranofin.[18]

SNP selection and genotyping

The region of *GZMB* plus the haplotype block upstream and downstream of the gene were tagged using the pairwise Tagger algorithm developed by de Bakker et al[21] and implemented in Haploview.[22] Two SNPs (rs8192917 and rs2236338) were known to be amino

acid–changing SNPs. One intergenic SNP, rs854350, had a significant association with RA susceptibility in the data set of the Wellcome Trust Case Control Consortium.[12] These 3 SNPs were force included. Pairwise-tagging SNPs were selected from the CEPH/CEU HapMap data set (phase II, release 21, NCBI build 35) using Haploview software (minor allele frequency [MAF] >0.05, pairwise $r^2 > 0.8$). Sixteen SNPs captured *GZMB*. Multiplex SNP arrays were designed using an Illumina GoldenGate platform, according to the protocols recommended by the manufacturer. One SNP could not be designed (rs1951601), but a good proxy (rs12433772; $r^2 = 0.90$) was typed instead. The final SNP selection and the linkage disequilibrium (LD) information are shown in Figure 1.

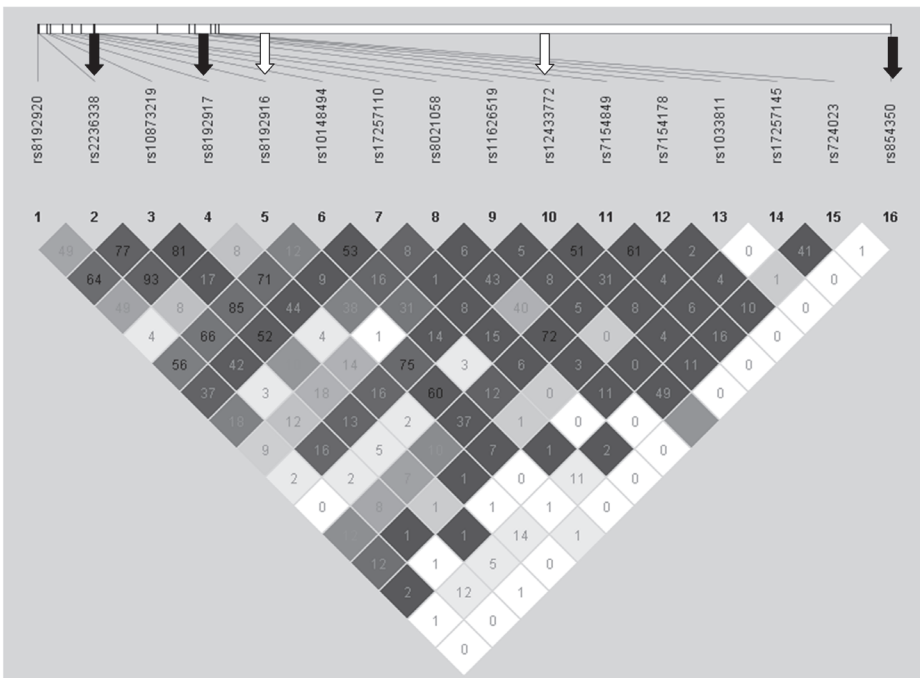


Figure 1 LD structure between of 16 tag-SNPs in GZMB.
The depicted data are from 600 Leiden RA-patients. The numbers present the r^2 between the SNPs. The colours refer to D´. Black arrows point out the amino acid changing SNPs, rs2236338 and rs8192917, and the susceptibility SNP of WTCCC, rs854350. These SNPs were forced to include. One SNP, rs1951601, could not be designed but a good proxy existed: rs1243372, r^2=0.90. One SNP, rs8192920, was not analyzed because of low success rate of typing. Significant SNPs in the analyses on the Leiden dataset are marked by a white arrow.

Software supplied by Illumina was used to automatically identify the genotypes. Each 96-well plate consisted of one positive and one negative control. Clusters were evaluated, and all doubtful calls were checked. After manually evaluating the spectra of each cluster, the genotypes were accepted, recalled, or rejected. At least 12% of the genotypes were

assessed in duplicate, with an error rate of <1% for all SNPs. One SNP, rs8192920, had a success rate of 75% and was therefore excluded from further analysis. The remaining SNPs were typed with a success rate of >95% (additional information is available from the corresponding author). None of the SNPs deviated from Hardy-Weinberg equilibrium ($P < 0.001$).

The SNP that was significantly associated with joint destruction in the first cohort was genotyped in the other 3 cohorts. The SNP was genotyped as a part of multiplex SNP arrays designed with Sequenom iPLEX, according to the protocols recommended by the manufacturer. Software supplied by the same manufacturer was used to automatically identify the genotypes. Each iPLEX consisted of at least 9 positive controls and 9 negative controls. All doubtful calls were checked manually; DNA samples in which >30% of the SNPs failed were excluded from analysis (n = 31). At least 5% of the genotypes were assessed in duplicate, with an error rate of <1%. The success rates were all >95%. None of the SNPs deviated from Hardy-Weinberg equilibrium.

Measuring and analyzing *GZMB* expression levels

The SNP that remained significant after meta-analysis was tested for its association with RNA expression of the genomic region of *GZMB*, by mapping expression quantitative trait locus (eQTL) on a data set representing peripheral blood samples obtained from 1,469 unrelated individuals.[23,24] RNA expression was tested using probes with a midpoint position <250 kb from the tested SNP, thus testing for a *cis* effect. Expression QTL effects were determined using Spearman's rank correlation coefficient. If an association was observed, it was determined whether other SNPs in the region (n = 583) had a stronger *cis*-eQTL effect. If this was the case, the first analysis was repeated using linear regression with adjustment for the strongest associating SNP, in an effort to determine the eQTL effect of the assessed SNPs independently of LD with the most strongly associated SNP. Genotypes were imputed with HapMap2 (release 24), using Impute version 2. Only SNPs with a MAF >5% and a Hardy-Weinberg *P* value greater than 0.0001 were included for analysis. Correction for multiple testing was performed by controlling the false discovery rate at 5%, permuting the gene expression labels 100 times as previously described.[25] Finally, to prevent false-positive findings due to primer polymorphisms, SNP–probe combinations were excluded from the *cis*-eQTL analysis when the 50-bp long expression probe mapped to a genomic location that contained a known SNP that was showing at least some LD ($r^2 > 0.1$) with the *cis* SNP.

Statistical analysis

Associations between genotypes and radiographic joint destruction were analyzed in 2 phases. First, an explorative analysis was performed in the Leiden EAC data set. In this data set, the tagged SNPs were tested both additively and recessively. Because phase 1 was an

explorative phase, no correction for multiple testing was applied, and SNPs with a *P* value less than or equal to 0.05 were studied in phase 2. For analyses involving the Leiden EAC, Groningen, and Lund data sets, a multivariate normal regression model for longitudinal data was used, with the radiographic score as the response variable. This method is used to analyze all repeated measurements at once and takes advantage of the correlation between these measurements. This model is similar to a linear mixed-effects model, but no random-effects model is added.[26] To model the correlation over time, a heterogeneous first-order autoregressive matrix was used, which assumes a stronger correlation for measurements obtained over a shorter period of time compared with those obtained over a longer period of time. The effect of time was entered as a factor in the model, to capture properly the mean response profile over time.

To test for an association with the rate of joint destruction, an analysis with the SNP and its interaction with time (as a linear variable) in the model was conducted. Adjustment variables were entered based on their univariate association with joint destruction. In the Leiden EAC data set, adjustments were made for age, sex, and the described treatment periods. The Groningen data set was adjusted for age and the period before or during 1990 and the period after 1990, as a proxy for DMARD therapy. The analysis of the Lund data set was adjusted for age only, because sex and treatment were not associated with joint destruction in this data set.

In the Sheffield data set, each patient had a set of radiographs of the hands and feet at one time point. To make the scores comparable with those of the other data sets, the estimated yearly progression rate was calculated.[27] This was achieved by dividing the total Larsen score by the number of disease-years at the time of radiography, resulting in an estimation of the rate of joint destruction. Information regarding disease duration at the time of radiography was available for 391 patients. The SNP association was tested in a linear regression analysis, with the log-transformed estimated yearly progression rate as the outcome variable. No adjustments were applied, because none of the tested variables was univariately significantly associated with joint destruction. The analyses were performed using SPSS version 17.0. In all data sets, the radiographic scores were log-transformed to obtain a normal distribution. Because the analyses were performed on the log scale, the resulting coefficient on the original scale indicates the fold increase in joint destruction per year of follow-up: over a follow-up period of n years, the coefficient increases to the power of n.

In the present study, the power to detect genetic effects is a function of the number of patients and the number of measurements per patient studied. As shown previously, the precision of the estimate increases steadily with increasing numbers of radiographs per patient.[28] All 3 data sets studied to verify the results obtained in phase 1 contained (individually and combined) fewer radiographs than the initial data set. Consequently, the power to replicate findings in each data set individually as well as in the 3 replication data

sets together could be limited due to the large number of radiographs in the discovery data set. Because of differences in study designs, the separate data sets could not be combined in one analysis directly. Therefore, the SNPs in each data set were tested separately, thereby taking advantage of the specific data set characteristics, and a meta-analysis of the results was performed subsequently to determine the association of the SNPs with the rate of joint destruction. Because the parameters in all data sets reflect the relative increase in the rate of joint destruction per year, the estimates of the individual data sets were pooled in a meta-analysis.[29] A fixed-effects meta-analysis[30] with inverse-variance weighting was performed using Stata version 10.1.

RESULTS

Phase 1 (SNP identification)

GZMB was tagged by 16 SNPs, one of which was not analyzed because of a low typing success rate. From the 15 analyzed SNPs, 2 (rs8192916 and rs12433772) were significantly associated with joint destruction in the Leiden EAC data set (Table 2). Patients homozygous for the minor allele of rs8192916 had an average 1.05-fold (range 1.02–1.08- fold) increase in the rate of joint destruction per year compared with the rate in the other patients ($P = 1.2 \times 10^{-3}$) (Figure 2). Because the estimated rate of progression increases by the power of the number of years, a coefficient of 1.05 per year resulted in a 1.41-fold increased rate of joint destruction over 7 years. SNP rs12433772 was highly linked to rs8192916, and because the effect of rs8192916 was more evident at the subsequent time points, this SNP was prioritized for the phase 2 analysis.

Table 2 SNPs in *Granzyme-B* associated with progression of joint destruction in Leiden RA-patients.

SNP	coordinate	Tested model	MAF	Coefficient	95% CI		*P*
rs8192916	24174200	ADD	0.42	1.01	1.00	1.03	0.11
		REC		1.05	1.02	1.08	1.2×10^{-3}
rs12433772	24190515	ADD	0.38	1.02	1.00	1.04	0.02
		REC		1.06	1.03	1.09	2.9×10^{-4}

MAF: minor allele frequency. 95%CI: 95% confidence interval

For the tagging process NCBI build 35, dbSNP b125 was applied. To make all coordinates in the paper consistent (SNPs and probes) NCBI build 36 was used to report the locations.

The coefficient of the additive test represents the relative increase in joint destruction per year per minor allele compared to no minor alleles. The coefficient of the recessive test represents the relative increase in joint destruction per year for two minor alleles compared to no one or no minor alleles on the normal scale. A coefficient of 1.05 per year indicates 5% higher rate of joint destruction; this implies that over a period of 7-years a 41% (1.05^7=1.41) higher rate of joint destruction is achieved.

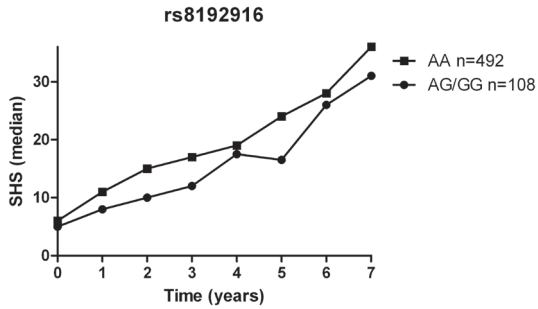


Figure 2 Median Sharp-van der Heijde scores over 7 follow-up years of Leiden RA patients with different rs8192916 genotypes.
Patients homozygous for the minor allele of rs8192916 had 1.05 (1.02-1.08 P=1.2x10^{-3}) fold rate of joint destruction per year than the other patients. This equals a 1.41 fold rate of joint destruction over a follow-up of 7 years.

Phase 2 (meta-analysis)

SNP rs8192916 was tested in a recessive model in all 3 replication data sets. As expected, due to insufficient power of the replication cohorts, the 95% confidence intervals (95% CIs) in each of the 3 cohorts separately all included 1 (Figure 3). An inverse-variance weighting meta-analysis was used to analyze rs8192916 in all 1,418 patients. SNP rs8192916 remained significant (P = 7.8 x 10^{-4}), thereby estimating a higher rate of joint destruction per year in patients homozygous for the minor allele compared with the other patients

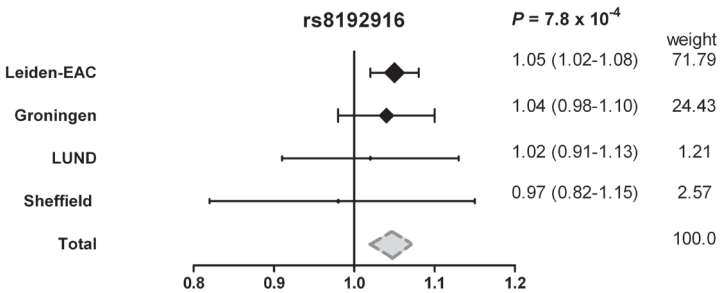


Figure 3 Depicted are the results of the analyses of rs8192916 of all four cohorts and of the final meta-analysis.
The effect sizes are the estimated relative progression rates per year for the presence of twice the minor allele compared to patients with only one or no minor allele.
The meta-analyses are based on a fixed effect model, which are applied to genetic studies to test whether there is statistically significant effect; generalisability of the effect is of less importance. As result of this choice, the effect size of the meta-analyses should be considered carefully. Consequently, this method is less suitable to estimate the effect size overall. Therefore, the estimated effect of the meta-analysis is depicted in gray.

(Figure 3). This association remained significant ($P = 1.2 \times 10^{-2}$) after conservative correction (Bonferroni adjustment) for testing 16 SNPs (the number of SNPs tested in phase 1).

When the association of rs8192916 with the rate of joint destruction was stratified for anti–citrullinated protein antibody (ACPA) status, the association was present in both subgroups (effect size 1.05 [95% CI 1.02–1.08], $P = 1.98 \times 10^{-3}$ and effect size 1.03 [95% CI 0.99–1.08], $P = 5.40 \times 10^{-2}$ for ACPA-negative and ACPA-positive patients, respectively).

Measuring and analyzing *GZMB* expression levels

To further substantiate the observed association of rs8192916 with joint destruction, the effect of rs8192916 on RNA expression was studied by *cis*-eQTL mapping in peripheral blood samples obtained from 1,469 unrelated individuals.[22]

SNP rs8192916 was significantly correlated with RNA expression of 4 different genes: *GZMB, CTSG, CBLN3,* and an unnamed gene with accession no. AK056368 ($P = 1.38 \times 10^{-5}$ to 6.14×10^{-6}) (Table 3). For all 4 genes, a SNP other than rs8192916 was more strongly correlated with RNA expression. To assess whether the SNP associated with joint destruction, rs8192916, had an effect on gene expression independently of the effect of the most strongly associated SNPs, the analyses were subsequently adjusted for the strongest SNP. After this correction was performed, rs8192916 was still associated with RNA expression of the *GZMB* probe ($P = 0.015$) (Table 3).

Table 3 Results of significant correlations of rs8192916 with *cis*-eQTL of RNA in peripheral blood.

SNP	Expressed Gene	Probe midpoint	eQTL significance	FDR	Strongest associating SNP* (*P-value*)	Corrected *P-value*[8]
rs8192916	GZMB	24171665	2.27×10^{-5}	3.03×10^{-5}	rs2236337 (3.98×10^{-119})	0.015
	CTSG	24113935	1.38×10^{-5}	1.38×10^{-5}	rs12878578 (8.91×10^{-63})	0.115
	CBLN3	23967077	2.27×10^{-5}	4.54×10^{-5}	rs2273629 (1.79×10^{-69})	0.057
	AK056368	24016093	6.14×10^{-6}	2.46×10^{-6}	rs12896086 (3.46×10^{-14})	0.752

Depicted information on location of probes is based on NCBI 36.3 build.

* Other SNPs in the region were stronger correlated with the RNA-expression, the strongest are summarized here.

[8] The association of rs8192916 with *cis*-eQTL was corrected for the strongest correlated SNP*.

Rs8192916 was correlated with the RNA-expression of several probes. Four probes, covering four different genes, were significantly correlated to rs8192916. These four correlations were further corrected for the SNPs* that correlated strongest with the RNA-expression.

DISCUSSION

The variance in joint destruction between patients with RA is considerable, and the mechanisms driving these differences are thus far scarcely understood. We performed a candidate gene study to investigate the association of genetic variants in *GZMB* with joint destruction. *GZMB* (14q11.2) was chosen as a candidate gene, because granzyme B is involved in inflammation, and data are emerging that granzyme B could be relevant to joint destruction in RA.[5-8,10,11] We tested the association of SNPs tagging *GZMB* with the rate of joint destruction in one data set and subsequently tested the significant SNP in 3 other data sets. Next, a meta-analysis combining the radiographic data of all 1,418 patients was performed. One SNP, rs8192916 (situated 9 kb upstream of *GZMB*), was observed to associate significantly with progression of joint destruction. The minor allele of rs8192916 was associated with a higher rate of joint destruction. We further observed that carrying this minor allele of rs8192916 was correlated with higher RNA expression of *GZMB* in whole blood. The present study uniquely combines 4 data sets representing patients with a similar European ethnicity who were treated in the prebiologic agent era. Hence, the radiographic progression rate of the patients studied here are more reflective of the natural course of RA compared with that of recently treated patients. In some data sets, patients were included over a wide time span; as treatment strategies changed over time, these patients received different treatments. Because different treatment regimens are associated with our outcome of interest (joint destruction), the analyses were adjusted for treatment, when relevant. Further studies on different patient populations as well as those treated with biologic agents would be informative.

Replication data sets are ideally larger than the initial data set; because the effect sizes in the discovery data set are likely to be upwardly biased, a smaller effect size is expected at a replication stage. Unfortunately, relatively few large prospective data sets exist in which both radiographs and DNA are available for conventionally treated patients. The number of patients and the number of radiographs in each data set separately were insufficient to allow well-powered analyses. Also, the replication data sets combined contained fewer radiographic measurements than were included in phase 1. Consequently, the data available for the phase 2 analysis were expected to be underpowered to replicate findings individually. Therefore, data were summarized in an inverse-variance weighting meta-analysis, which showed a significant result for rs8192916.

To further substantiate the findings for *GZMB*, RNA expression was studied and revealed a strong signal for rs8192916. To confirm that this correlation was not driven by a linked SNP, we sought to identify other SNPs in the region that also associate with RNA expression. SNP rs2236337 had the strongest correlation with *GZMB* expression. However, LD between rs2236337 and rs8192916 was low (r^2 = 0.08). Moreover, the fact that after

correction for this SNP, rs8192916 remained significant suggests that the correlation of rs8192916 with *GZMB* expression is likely independent of the influence of rs2236337.

In the current study, *GZMB* was tagged by pairwise SNP selection, with an MAF of >0.05 and a pairwise r^2 value of >0.8. Inherent to this method, rare variants are not selected. Therefore, based on the results of the current study, we cannot exclude the possibility that other variants in *GZMB* also associate with joint destruction.

Granzyme B, which is released by NK cells and T lymphocytes, can induce cell death in harmful cells, such as those that are virally infected or malignant.[31] Recent findings have suggested a role for granzyme B in RA joint destruction as well; the number of granzyme B–positive cells is increased in synovium as well as among chondrocytes at the site of pannus lesions.[8,32]

The findings of the current study are consistent with those of previous studies. Possibly, the minor variant of rs8291916, or a genetic variant linked to this SNP, induces higher expression of granzyme B, leading to more apoptosis in chondrocytes and therefore more joint destruction. This would explain the previously observed association of higher serum granzyme B levels in patients with erosive disease.[11] Another explanation could be the role of granzyme B in propagation of the inflammatory response; additional studies are needed to determine this.

In conclusion, using a candidate gene approach evaluating patients in 4 different cohorts, we observed associations between rs8192916 and an increased rate of joint destruction in RA and between rs8192916 and expression profiles of *GZMB*. Although evaluation of rs8192916 at the protein level was not performed, these data suggest that carriage of the risk allele may affect the function of *GZMB*.

REFERENCE LIST

1. Van der Heijde DM, van Riel PL, van Leeuwen MA, et al. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis: a prospective follow-up study of 147 patients. *Br J Rheumatol* 1992;31:519–25.
2. De Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes: what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011;50:93–100.
3. Van der Helm-van Mil AH, Kern M, Gregersen PK, et al. Variation in radiologic joint destruction in rheumatoid arthritis differs between monozygotic and dizygotic twins and pairs of unrelated patients. *Arthritis Rheum* 2006;54:2028–30.
4. Knevel R, Grondal G, Huizinga TW, et al. Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study. *Ann Rheum Dis* 2012;71:707–9.
5. Trapani JA, Davis J, Sutton VR, et al. Proapoptotic functions of cytotoxic lymphocyte granule constituents in vitro and in vivo. *Curr Opin Immunol* 2000;12:323–9.
6. Lord SJ, Rajotte RV, Korbutt GS, et al. Granzyme B: a natural born killer. *Immunol Rev* 2003;193:31–8.
7. Froelich CJ, Zhang X, Turbov J, et al. Human granzyme B degrades aggrecan proteoglycan in matrix synthesized by chondrocytes. *J Immunol* 1993;151:7161–71.
8. Ronday HK, van der Laan WH, Tak PP, et al. Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. *Rheumatology (Oxford)* 2001;40:55–61.
9. Korb-Pap A, Stratis A, Muhlenberg K, et al. Early structural changes in cartilage and bone are required for the attachment and invasion of inflamed synovial tissue during destructive inflammatory arthritis. *Ann Rheum Dis* 2012;71:1004–11.
10. Tak PP, Spaeny-Dekking L, Kraan MC, et al. The levels of soluble granzyme A and B are elevated in plasma and synovial fluid of patients with rheumatoid arthritis (RA). *Clin Exp Immunol* 1999;116:366–70.
11. Goldbach-Mansky R, Suson S, Wesley R, et al. Raised granzyme B levels are associated with erosions in patients with early rheumatoid factor positive rheumatoid arthritis. *Ann Rheum Dis* 2005;64:715–21.
12. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
13. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
14. Ropes MW, Bennett GA, Cobb S, et al. 1958 revision of diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 1958;9:175–6.
15. Van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000;27:261–3.
16. Mewar D, Coote A, Moore DJ, et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. *Arthritis Res Ther* 2006;8:R128.
17. Rau R, Herborn G. A modified version of Larsen's scoring method to assess radiologic changes in rheumatoid arthritis. *J Rheumatol* 1995;22:1976–82.
18. Fex E, Jonsson K, Johnson U, et al. Development of radiographic damage during the first 5-6 yr of rheumatoid arthritis: a prospective follow-up study of a Swedish cohort. *Br J Rheumatol* 1996;35:1106–15.
19. Lindqvist E, Jonsson K, Saxne T, et al. Course of radiographic damage over 10 years in a cohort with early rheumatoid arthritis. *Ann Rheum Dis* 2003;62:611–6.
20. Larsen A, Dale K. Standardised radiological evaluation of rheumatoid arthritis in therapeutic trials. In: Dumonde DC, Jasani MK, eds. The recognition of anti-rheumatic drugs. *Lancaster (UK): MTP Press;* 1978. p. 285–92.

21. De Bakker PI, Yelensky R, Pe'er I, et al. Efficiency and power in genetic association studies. *Nat Genet* 2005;37:1217–23.
22. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21: 263–5.
23. Dubois PC, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet* 2010;42:295–302.
24. Fehrmann RS, Jansen RC, Veldink JH, et al. Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. *PLoS Genet* 2011;7:e1002197.
25. Breitling R, Li Y, Tesson BM, et al. Genetical genomics: spotlight on QTL hotspots. *PLoS Genet* 2008;4:e1000232.
26. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis. *New York: John Wiley & Sons*; 2004.
27. Strand V, Landewe R, van der Heijde D. Using estimated yearly progression rates to compare radiographic data across recent randomised controlled trials in rheumatoid arthritis. *Ann Rheum Dis* 2002;61 Suppl 2:ii64–6.
28. Van der Helm-van Mil AH, Knevel R, van der Heijde D, et al. How to avoid phenotypic misclassification in using joint destruction as an outcome measure for rheumatoid arthritis? *Rheumatology (Oxford)* 2010;49:1429–35.
29. Lassere M. Pooled metaanalysis of radiographic progression: comparison of Sharp and Larsen methods. *J Rheumatol* 2000;27: 269–75.
30. Lebrec JJ, Stijnen T, van Houwelingen HC. Dealing with heterogeneity between cohorts in genomewide SNP association studies. *Stat Appl Genet Mol Biol* 2010;9:Article 8.
31. Trapani JA, Sutton VR. Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Curr Opin Immunol* 2003;15:533–43.
32. Saito S, Murakoshi K, Kotake S, Kamatani N, et al. Granzyme B induces apoptosis of chondrocytes with natural killer cell-like cytotoxicity in rheumatoid arthritis. *J Rheumatol* 2008;35: 1932–43.

8

Serum Pyridinoline Levels and Prediction of Severity of Joint Destruction in Rheumatoid Arthritis

A. Krabben, R. Knevel, T. W. J. Huizinga, G. Cavet,
A. H. M. van der Helm-van Mil

Journal of Rheumatology, 2013 August;40(8):1303-1306.

ABSTRACT

Objective

Previous studies indicated that pyridinoline, a collagen crosslink in cartilage and bone, might be a good marker to predict joint destruction in patients with rheumatoid arthritis (RA), although large prospective studies are lacking. We evaluated the predictive value of serum pyridinoline levels for joint destruction, both at baseline for long term prediction and during the disease course for near-term prediction.

Methods

Patients with early RA from the Leiden Early Arthritis Clinic were studied. Radiographs at baseline and yearly during 7 years of follow-up were scored according to the Sharp-van der Heijde Scoring (SHS) method. Pyridinoline serum levels at baseline and during follow-up were measured by ELISA. The association between baseline pyridinoline levels and difference in SHS over 7 years was tested, with a multivariate normal regression model. Second, the association between pyridinoline levels determined during the disease course and progression of SHS over the next year was tested with a multivariable linear regression analysis.

Results

Studying baseline pyridinoline serum levels in 437 patients revealed that the mean SHS over 7 years was 6% higher for every higher pyridinoline level (nmol/l) at baseline ($p = 0.001$). Subsequently, during follow-up ($n = 184$ patients) the progression in SHS in the upcoming year was 17% higher for every higher nmol/l pyridinoline level ($p = 0.001$). The area under the receiver-operation characteristic curve for rapid radiological progression was 0.59.

Conclusion

Increased pyridinoline serum levels, both at baseline and during the disease course, are associated with more severe joint destruction during the coming year(s), although the predictive accuracy as a sole predictor was moderate.

INTRODUCTION

Predicting an individual patient's future disease severity is required for decisions for individualized treatment. At disease onset it can guide the initial treatment strategy and during the disease course it may guide treatment adaptations. The severity of rheumatoid arthritis (RA) is often measured using the extent of joint destruction. At present, only part of the variance in joint destruction is explained by currently known risk factors, which consist mainly of autoantibodies and markers of inflammation.[1] Markers of cartilage or bone metabolism have been studied to a lesser extent. Studies have found evidence that C-terminal crosslinking telopeptide of type I collagen (CTX-I) and C-terminal crosslinking telopeptide of type II collagen (CTX-II)[2] were associated with radiologic progression. Pyridinoline is a major crosslink of collagen in cartilage and is also present in collagen of bone and other tissue as synovium. Based on initial studies it can be presumed that pyridinoline might be a good marker for RA as well. Pyridinoline levels are increased in patients with RA compared to healthy persons or to patients with other rheumatologic diagnoses.[3-6] In addition, some studies indicate that pyridinoline levels are higher in cases of active or severe RA.[3,4,7-9] Most previous studies were performed on urine levels of pyridinoline.

To date no large longitudinal studies on serum pyridinoline levels and severity of joint destruction in RA have been performed. We evaluated the predictive value of serum pyridinoline levels for future joint destruction, both at baseline for longterm prediction and during the course of RA for more near-term prediction of disease severity.

MATERIALS AND METHODS

Patients

Patients included in the Leiden Early Arthritis Clinic (EAC) cohort that fulfilled the 1987 American College of Rheumatology criteria for RA at baseline or during the first year of disease were studied. The Leiden EAC is a large, prospective cohort.[1] Briefly, patients were referred by general practitioners when arthritis was suspected and were included in the EAC cohort if arthritis was confirmed on physical examination and symptom duration was < 2 years. At inclusion, patients were queried about their joint symptoms and underwent examination. At baseline and at the yearly follow-up visits, blood samples were taken for routine diagnostic laboratory screening and serum was stored at –70°C. Radiographs of hands and feet, taken at baseline and yearly thereafter during 7 years of follow-up, were scored according to the Sharp-van der Heijde Scoring (SHS) method with known time order by an experienced reader, blinded to any clinical information. The within-reader ICC was 0.91. Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee.

Pyridinoline levels

Pyridinoline serum levels were measured by ELISA (QuickVue; Quidel Corp.), according to the manufacturer's instructions, using stored baseline and follow-up serum samples. All baseline and all follow-up ELISA were done at the same point in time. The intra-assay coefficient of variation (CV) was 6.8% and the inter-assay CV was 11.1%. The limit of detection was 0.5 nmol/l (lower limit of quantification 0.5 nmol/l and upper limit of quantification 12 nmol/l).

Statistical analyses

To approximate a normal distribution, all radiological data were log-transformed before analyses. First, associations between baseline pyridinoline levels and log-transformed radiographic joint destruction over 7 years were analyzed using a multivariate normal regression model with radiological score as response variable, testing for a constant difference in SHS. This method analyzes all repeated measurements at once and takes advantage of the correlation between these measurements. The effect of time was entered as a factor in the model, to properly capture the mean response profile over time.[10] All analyses were adjusted for age, sex, and treatment strategy. The treatment strategies differed according to the inclusion period, which was used as a proxy for treatment strategy in the analyses. Patients included in 1993–95 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996–98 were initially treated with chloroquine or salazopyrine, and patients included after 1999 were promptly treated with methotrexate or salazopyrine. Since the baseline SHS is included in the repeated measurement analyses as the response variable, baseline SHS was not also included as an adjustment factor. Next, additional adjustments were made including other known risk factors for progression of joint destruction: anti-cyclic citrullinated peptide (CCP) antibody status, C-reactive protein (CRP) levels, body mass index (BMI), and smoking. The analyses were repeated for the total erosion score and the total joint space narrowing score as outcomes, adjusted for age, sex, and treatment.

The association between pyridinoline levels determined during the disease course and progression of joint damage during the next year was studied with a multivariable linear regression analysis with log-transformed delta SHS over the upcoming year as response variable, adjusting for age, sex, treatment, and disease duration. Thereafter, additional adjustments were made for CCP-positivity and CRP level. The first analysis with pyridinoline levels during disease course were also repeated with total joint space narrowing score and erosion score as outcomes.

To evaluate the prognostic accuracy for clinical practice, the positive and negative likelihood ratios (LR) and area under the receiver-operator characteristic (ROC) curve (AUC) were determined. Therefore pyridinoline levels and radiographic progression were recoded as binary variables. Radiologic data were categorized using a definition of rapid radiographic

progression (increase of SHS ≥ 5 after 1 year) as reported.[11] This was done at baseline and during the disease course. The cutoff point to categorize pyridinoline levels was determined based on the optimal maximum from the ROC curve, with rapid radiological progression (RRP) as outcome. Finally, the prognostic accuracy was determined for pyridinoline levels on RRP and any progression in joint destruction during the next year (an increase of ≥ 1 SHS in 1 year). Correlations between CRP and pyridinoline levels were determined with Spearman's correlation coefficients. Analyses were performed using SPSS version 20.0. P values < 0.05 were considered significant.

RESULTS

Baseline serum pyridinoline levels

Serum pyridinoline levels were determined in 437 patients with early RA, who had median symptom duration of 19 weeks. Patient characteristics are provided in Table 1. The median serum level was 4.7 nmol/l (range 3.8–6.0). These baseline levels were significantly associated with the severity of joint damage over 7 years. For every nmol/l increase in pyridinoline level at baseline, patients had 6% higher total SHS (β = 1.06, p = 0.001; Figure 1A). After adjustment for age, sex, and treatment and also for known risk factors such as anti-CCP positivity, CRP levels, BMI, and smoking, the association between serum pyridinoline level and joint destruction remained significant (β = 1.06, p = 0.044).

The total SHS is a composite of the total erosion score and joint space narrowing score. In order to determine whether serum pyridinoline was associated with bone or cartilage destruction in particular, analyses were repeated on these 2 sub-scores. Significant associations were observed between serum pyridinoline levels at baseline and both the severity of erosions and joint space narrowing over time (β = 1.06, p = 0.025, and β = 1.05, p = 0.002, respectively).

Table 1 Characteristics of the RA-patients studied

	Patients with baseline serum samples (n=437)	Patients with follow-up serum samples (n=184)
Age, mean ± SD, years	57 ± 16	56 ± 14
Female sex, n (%)	307 (70)	122 (66)
ACPA positivity, n (%)	227 (52)	121 (66)
CRP level, median (IQR), mg/dL	1.8 (0.8-4.0)	0.7 (0.3-1.4)
SHS, median (IQR)	5 (2-11)	23 (11-46)

For baseline samples, the patient characteristics at baseline are presented. For the follow-up samples, the patient characteristics at the time of serum sampling are presented.

Figure 1A Baseline serum pyridinoline levels and predicted joint destruction over 7 years

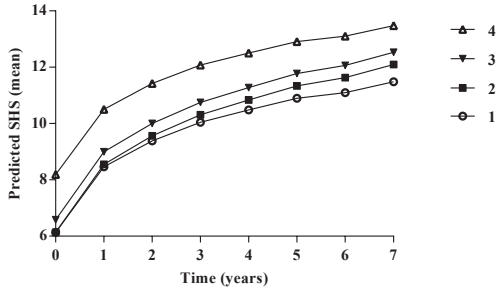


Figure 1B Correlation between baseline and follow-up pyridinoline levels

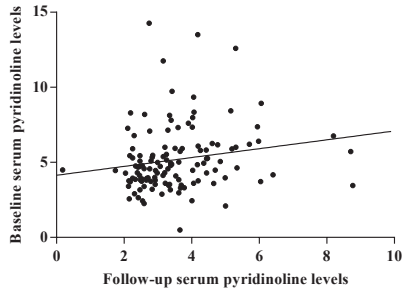


Figure 1C Pyridinoline serum levels during disease course and joint destruction the upcoming year

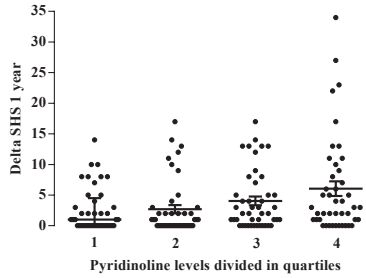


Figure 1 Association between pyridinoline baseline serum levels with the severity of joint destruction over 7 years (A), correlation between baseline serum levels with follow-up serum levels (B), and association of pyridinoline follow-up serum levels with progression of joint damage during the next year (C).
Ad Figure 1A Depicted is the predicted SHS from a multivariable normal regression model, with continuous pyridinoline levels, age, gender and treatment, on the y-axis and time on the x-axis. Pyridinoline serum levels were analyzed at a continuous level, but divided in quartiles to depict in a graph. The first quartile represents the lowest 25% of pyridinoline levels, the second quartile the highest 25% of the lowest 50%, the third quartile the lowest 25% of the highest 50% and the forth quartiles represents the highest quartile of pyridinoline levels.
Ad Figure 1C Pyridinoline serum levels were analyzed as a continuous variable but divided in quartiles to depict in a graph. The first quartile represents the lowest 25% of pyridinoline levels, the second quartile the highest 25% of the lowest 50%, the third quartile the lowest 25% of the highest 50% and the forth quartile represents the highest quartile of pyridinoline levels. Horizontal lines indicate the median, with two-sided inter quartile range bars.

Serum pyridinoline levels during the disease course

Follow-up serum samples of 184 patients with RA were studied; the mean disease duration at serum sampling was 4 years (± 2 yrs). The median serum level was 3.3 nmol/l (range 2.6–4.2). Pyridinoline levels measured at baseline and during the disease course were correlated (r_s = 0.251, p = 0.005; Figure 1B). Subsequently, the association between serum

pyridinoline levels and near-term increase in SHS score over the next year was evaluated. After adjustment for age, sex, treatment, and disease duration it was observed that for every nmol/l increase in pyridinoline level, the progression in SHS over the upcoming year was 17% higher ($\beta = 1.17$, $p = 0.001$; Figure 1C). Also including adjustments for anti-CCP positivity and CRP level, the association between pyridinoline level and progression in SHS over the upcoming year remained significant ($\beta = 1.15$, $p = 0.003$).

Sub-analyses on the erosion or joint space narrowing scores as outcomes also here yielded significant results for the severity of bone and cartilage destruction ($\beta = 1.10$, $p = 0.014$, and $\beta = 1.10$, $p = 0.025$, respectively).

Prognostic accuracy

The optimal maximum of baseline serum pyridinoline levels on the ROC curve with RRP as outcome was 4.7480 nmol/l. Patients with a baseline pyridinoline level ≥ 4.8 nmol/l had a positive LR of 1.30 for RRP (increase in SHS ≥ 5) during the first year. The negative LR was 0.77 and AUC was 0.59. When taking any progression in joint destruction in the first year as outcome, positive LR was 1.25, negative LR was 0.83, and AUC was 0.56.

The optimal maximum of follow-up serum pyridinoline levels on the ROC curve with RRP as outcome was 3.3983 nmol/l. Patients with RA who during the disease course had a pyridinoline level ≥ 3.4 nmol/l had a positive LR of 1.49 for RRP (SHS increase ≥ 5) during the next year. The negative LR was 0.67 and AUC was 0.61. Taking SHS ≥ 1 during the next year as the outcome, the positive LR was 2.00, negative LR was 0.61, and AUC was 0.64.

DISCUSSION

We investigated the predictive value of serum pyridinoline levels for future joint destruction and observed that increased pyridinoline levels were significantly associated with more severe joint destruction. This applied to baseline serum levels and joint damage over 7 years as well as to serum levels determined during the disease course and joint destruction during the upcoming year.

The association between pyridinoline and severity of joint damage was observed to be independent of the association of other factors (such as anti-CCP or CRP) with progression of joint damage. Although in the multivariable analyses serum pyridinoline was found to be an independent risk factor, the positive LR and AUC were moderate. This indicates that the serum pyridinoline level alone is insufficient for adequate prediction, but that it will be valuable to include serum pyridinoline in combined predictive models.

Intriguingly, serum levels taken at baseline and during the disease course were correlated. Further, both serum levels were associated with more severe joint destruction over time. Together these data may suggest that patients with more severe radiological progression

have increased serum pyridinoline levels at the start of and throughout the disease course. Because treatment effect may have influenced the outcome measures, all analyses were adjusted for differences in treatment.

The strengths of our study are the large group of patients with RA and the availability of high-quality long-term follow-up data. A limitation could be the lack of measured deoxypyridinoline. Deoxypyridinoline exists only in bone, and pyridinoline in both cartilage and bone. Hence the ratio of these markers can be used to determine whether serum pyridinoline was derived from breakdown of cartilage or bone. To make this distinction we performed sub-analyses on pyridinoline levels in relation to joint space narrowing and erosions. These showed that associations were present for both cartilage loss and bone damage.

Another limitation is that our analyses were based on patients from a single cohort. Additional large-scale longitudinal studies on the prognostic value of serum pyridinoline levels are needed to replicate our findings and to establish the predictive value of this biomarker.

Increased serum pyridinoline levels are of additive value to known serological risk factors for future joint damage in patients with RA, both at baseline and during the disease course. A combination of multiple biomarkers with other risk factors most likely will be required to make adequate predictions.

REFERENCE LIST

1. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes — what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology* 2011;50:93-100.
2. Garnero P, Landewe R, Boers M, et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. *Arthritis Rheum* 2002;46:2847-56.
3. Astbury C, Bird HA, McLaren AM, et al. Urinary excretion of pyridinium crosslinks of collagen correlated with joint damage in arthritis. *Br J Rheumatol* 1994;33:11-5.
4. Black D, Marabani M, Sturrock RD, et al. Urinary excretion of the hydroxypyridinium crosslinks of collagen in patients with rheumatoid arthritis. *Ann Rheum Dis* 1989;48:641-4.
5. Seibel MJ, Duncan A, Robins SP. Urinary hydroxy-pyridinium crosslinks provide indices of cartilage and bone involvement in arthritic diseases. *J Rheumatol* 1989;16:964-70.
6. Muller A, Jakob K, Hein GE. Evaluation of free and peptide bound collagen crosslink excretion in different skeletal diseases. *Ann Rheum Dis* 2003;62:65-7.
7. Le Loët X, Brazier M, Mejjad O, et al. Serum IgA rheumatoid factor and pyridinoline in very early arthritis as predictors of erosion(s) at two years: A simple model of prediction from a conservatively treated community-based inception cohort. *Arthritis Care Res* 2010;62:1739-47.
8. Jensen T, Klarlund M, Hansen M, et al. Connective tissue metabolism in patients with unclassified polyarthritis and early rheumatoid arthritis. Relationship to disease activity, bone mineral density, and radiographic outcome. *J Rheumatol* 2004;31:1698-708.
9. Hein G, Franke S, Muller A, et al. The determination of pyridinium crosslinks in urine and serum as a possible marker of cartilage degradation in rheumatoid arthritis. *Clin Rheumatol* 1997;16:167-72.
10. Knevel R, Krabben A, Brouwer E, et al. Genetic variants in IL15 associate with progression of joint destruction in rheumatoid arthritis: a multicohort study. *Ann Rheum Dis* 2012;71:1651-7.
11. Visser K, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, et al. A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: Post hoc analyses from the BeSt study. *Ann Rheum Dis* 2010;69:1333-7.

PART 3

MRI in patients with arthralgia and early arthritis

9

Magnetic Resonance Imaging of hand and foot joints of patients with ACPA positive arthralgia without clinical arthritis

A. Krabben, W. Stomp, D. van der Heijde,
J. A. B. van Nies, J. L. Bloem, T. W. J. Huizinga, M. Reijnierse,
A. H. M. van der Helm-van Mil

Annals of the Rheumatic Diseases, 2013 September;72(9):1540-1544.

ABSTRACT

Background

Anti-citrullinated peptide antibodies (ACPA) and acute phase reactants may be increased before arthritis becomes clinically detectable, suggesting that the processes underlying rheumatoid arthritis (RA) start pre-clinically. Whether local inflammation occurs in the preclinical phase is unknown. Therefore, we studied the small joints of ACPA positive arthralgia patients for local subclinical inflammation.

Methods

Imaging was performed using 1.5 T extremity MRI. Painful hand or foot joints of 21 ACPA positive arthralgia patients without clinical arthritis were imaged. For comparison, hand and foot joints of 22 ACPA positive RA patients and 19 symptom free controls were studied. Within ACPA positive arthralgia patients, painful and symptom free joint regions were imaged. Scoring was performed according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) Rheumatoid Arthritis Magnetic Resonance Imaging Scoring (RAMRIS) method. Analyses were performed on joint region level and focused on inflammation (synovitis plus bone marrow oedema).

Results

The mean combined inflammation scores of the metacarpophalangeal/proximal interphalangeal joints of controls, painful joints of ACPA positive arthralgia patients and ACPA positive RA patients were 0.1, 0.7 and 3.7, respectively ($p<0.001$). Likewise, the mean combined inflammation scores of the wrist were 0.9, 2.3 and 10.3, respectively ($p<0.001$) and that of the metatarsophalangeal joints 0.5, 0.9 and 3.8, respectively ($p=0.10$). At the MCP joints, the combined inflammation score was significantly correlated with C reactive protein and erythrocyte sedimentation rate levels ($r_s=0.83$ and $r_s=0.78$, respectively)

Conclusions

The present data suggest that local subclinical inflammation occurs in ACPA positive arthralgia patients.

INTRODUCTION

Recent studies have shown that anti-citrullinated peptide antibodies (ACPA) can be detected in the serum of ACPA positive rheumatoid arthritis (RA) patients years before arthritis becomes clinically detectable.[1] C reactive protein (CRP), cytokines and bone degradation markers have also been found to be elevated in this phase,[2] suggesting that the processes underlying the development of RA may start long before clinical arthritis occurs.

Therefore, detailed studies on inflammation in the preclinical phase may enhance our understanding of the development of ACPA positive RA.[3]

It is not yet known whether local inflammation occurs in small joints in the preclinical phase. A recent MRI study on knee joints of 13 ACPA positive arthralgia patients showed no subclinical inflammation.[4] However, ACPA positive RA probably does not start in the knee joints, leaving unanswered the question of whether local inflammation is present in the preclinical phase of ACPA positive RA.

This study aimed to investigate whether there is subclinical inflammation in painful metacarpophalangeal (MCP) joints, the wrist or metatarsophalangeal (MTP) joints in ACPA positive arthralgia patients. To this end, MRI scans of painful small joints of ACPA positive arthralgia patients were compared with scans of small joints of ACPA positive RA patients and of symptom free controls. Then, the results of the MRI scans of painful and symptom free joints were compared within ACPA positive arthralgia patients.

METHODS

Subjects

Three groups were studied. The first group comprised ACPA positive (anti-CCP2, Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands) patients with painful hand or foot joints but without clinical arthritis, who were recruited from the rheumatological outpatient clinic in Leiden University Medical Center between May 2011 and July 2012. In total, 25 ACPA positive arthralgia patients were recruited; three patients were excluded as clinically detectable arthritis was observed by a rheumatologist on the day of the MRI and one patient had an MRI artefact due to a metal fragment in his hand, leaving 21 patients for evaluation. Per patient, the region of the (most) painful joints (proximal interphalangeal (PIP) 2–5, MCP 2–5, wrist or MTP 1–5) was imaged. To allow comparisons within patients, the symptom free contralateral side was also scanned. When both sides were painful, another non-painful region was scanned (eg, in case of arthralgia of MCP joints at both sides, symptom free MTP joints were scanned). The regions that were scanned in ACPA positive arthralgia patients were not fixed in order to allow the flexibility to scan painful joints as well as symptom free joints in every patient. The second group of subjects were 22, 1987

American College of Rheumatology criteria positive ACPA positive early RA patients. These patients were included in the early arthritis cohort (EAC) Leiden between August 2010 and July 2012. In these RA patients, MRI of the MCP, wrist and MTP joints at the most painful side was performed. The third group comprised 19 healthy subjects without joint complaints, who underwent MRI of the MCP, wrist and MTP joints at the dominant side. Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee.

MRI

Imaging was performed on an ONI-MSK-Extreme 1.5 T extremity MRI (GE, Wisconsin, USA). Acquired sequences were T1 weighted fast spin echo sequences, T2 weighted fast spin echo sequences with fat saturation (both coronal plane), and after intravenous gadolinium contrast (0.1 mmol/kg), T1 weighted fast spin echo sequences with fat saturation in the coronal and axial planes. The field of view was 100 mm for all sequences. For ethical reasons, contrast was not administered in healthy controls. MRI scoring was performed by two trained readers according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) Rheumatoid Arthritis MRI Scoring (RAMRIS) method, evaluating erosions, bone marrow oedema and synovitis.[5,6] The mean scores of the two readers were analyzed (within reader intra-class correlation coefficient total RAMRIS score 0.98 and 0.83, and between reader intra-class correlation coefficient 0.82).

Analyses

Analyses were performed per different joint regions. The 'combined inflammation score' (synovitis plus bone marrow oedema) was analyzed using Kruskal–Wallis tests or Mann–Whitney U tests because of non-normally distributed data. The correlation between the painful and symptom free joints in ACPA positive arthralgia patients was tested with Spearman's correlation. SPSS V.20.0 was used. A p value<0.05 was considered significant.

RESULTS

Baseline characteristics of the ACPA positive arthralgia patients, ACPA positive RA patients and symptom free controls are presented in table 1. The arthralgic joint regions in the ACPA positive arthralgia patients were: MCP joints (n=4), PIP joints (n=4), wrist (n=3) and MTP joints (n=10) (see online supplementary table S1 for an overview of the scanned regions).

Given the study question, we specifically studied the inflammatory markers bone marrow oedema and synovitis (summed in the combined inflammation score) without the erosion score. In the wrist joints, the combined inflammation scores of the symptom free controls, ACPA positive arthralgia and ACPA positive RA patients were 0.9, 2.3 and 10.3,

Table 1 Patient characteristics

	Symptom-free Controls (n=19)	ACPA-positive arthralgia (n=21)	ACPA-positive RA (n=22)
Age, mean ± SD, years	46.2 ± 11.8	47.9 ± 7.9	53.7 ± 15.9
Female sex, n (%)	15 (78.9)	17 (81.0)	12 (54.5)
Symptom duration at the MRI, median (IQR), weeks	n/a	34.5 (15.3-114.5)	20 (11-37)
Onset of symptoms			
(sub) acute	n/a	6 (28.6)	7 (31.8)
gradual	n/a	15 (71.4)	15 (68.2)
Morning stiffness, median (IQR), minutes	n/a	30 (5-60)	60 (45-120)
Swollen joint count, median (IQR)	0	0	5 (3-12)
Tender joint count, median (IQR)	0	2 (1-3)	6 (5-11)
ACPA level, median (IQR)	n/a	326 (94-340)	182 (87-324.3)
RF positivity, n (%)	n/a	15 (71.4)	19 (86.4)
RF level, median (IQR)	n/a	22 (10.5-120)	20 (11.5-91)

Except where indicated otherwise, values are the number (%) of patients.
Interquartile range (IQR). Standard deviation (SD).
68 tender joint count and 66 swollen joint count were performed.
n/a: not applicable
Symptom duration refers to the period between the first symptom onset of any joint and the MRI date.

respectively ($p<0.001$). The combined inflammation scores of the MCP/PIP joints were 0.1, 0.7 and 3.7, respectively ($p<0.001$), and the combined inflammation score of the MTP joints 0.5, 0.9 and 3.8, respectively ($p=0.10$). When comparing ACPA positive arthralgia patients and symptom free controls, the combined inflammation scores were significantly higher in the wrist joints of ACPA positive arthralgia patients ($p=0.02$), but not in the MCP/PIP and MTP joints ($p=0.06$ and $p=0.32$, respectively) (figure 1A–C).

Subsequently, we evaluated all MRI features separately. In the wrist, MCP/PIP and MTP joints, a gradual increase was observed in all three features when comparing symptom free controls, ACPA positive arthralgia patients and ACPA positive RA patients (figure 1D–F).

Next the correlation between local and systemic inflammation in ACPA positive arthralgia patients was determined, revealing an association between both CRP and erythrocyte sedimentation rate (ESR) and the combined inflammation score of the MCP joints ($r_s=0.83$, $p=0.01$ and $r_s=0.78$, $p=0.02$, respectively). ACPA or rheumatoid factor levels were not correlated with MRI combined inflammation scores in the present study.

Thus far we studied the painful joints of ACPA positive arthralgia patients. Within the ACPA positive arthralgia patients, the mean combined inflammation score in the painful joints was 1.0 and in the symptom free joints 1.2. In the 21 patients studied, we did not observe a significant correlation between these two joint regions ($r_s=0.32$ and $p=0.16$).

Figure 1A Combined inflammation score wrist joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

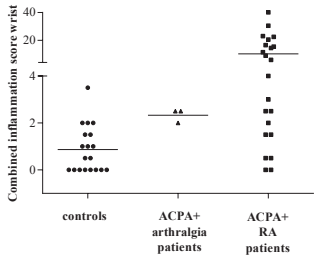


Figure 1B Combined inflammation score MCP/PIP joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

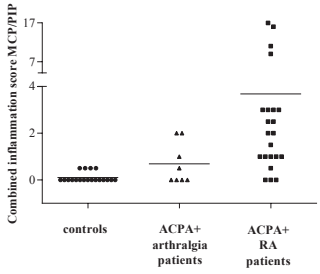


Figure 1C Combined inflammation score MTP joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

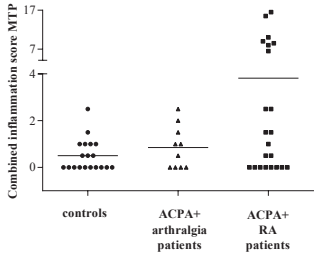


Figure 1D Mean RAMRIS subscores in wrist joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

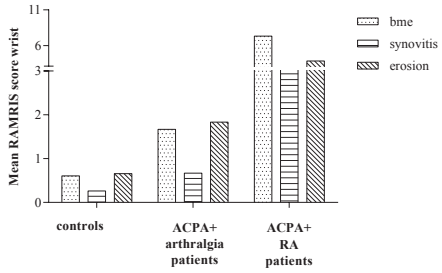


Figure 1E Mean RAMRIS subscores in MCP/PIP joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

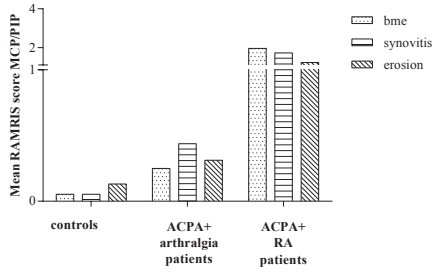


Figure 1F Mean RAMRIS subscores in MTP joints in; controls, ACPA+ arthralgia patients and ACPA+ 1987-RA patients

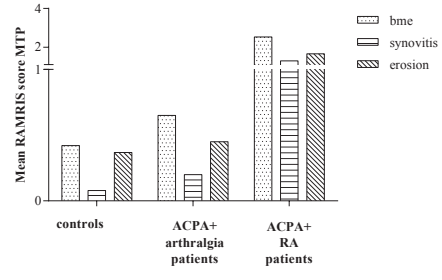


Figure 1 Combined inflammation scores (bone marrow edema plus synovitis) (A-C) and separate scores for bone marrow edema, synovitis and erosion (D-F) in symptom-free healthy controls, ACPA-positive arthralgia-patients and ACPA-positive RA-patients per joint region.

All scores are mean scores of two readers. Horizontal lines represent mean scores. The y-axes were splitted as the RA-patients had higher scores than the ACPA-positive arthralgia patients and healthy controls. The mean combined inflammation-scores of the wrist-joints of the controls, the painful joints of ACPA-positive arthralgia-patients and ACPA-positive RA-patients were 0.9, 2.3, 10.3, respectively ($p<0.001$). The mean combined inflammation-scores of the MCP/PIP-joints were 0.1, 0.7 and 3.7 respectively ($p<0.001$) and that of the MTP-joints 0.5, 0.9, 3.8, respectively ($p=0.10$). Similarly, for these three groups the mean erosion scores were 0.7, 1.8 and 3.8 in the wrist 0.1, 0.3 and 1.2 in the MCP/PIP 0.4, 0.5 and 1.7 and in the MTP-joints respectively.

In the follow-up period (mean 9 months (range 1–15 months)), 12 arthralgia patients developed RA according to the 2010 American College of Rheumatology/European League Against Rheumatism RA criteria. The combined inflammation scores between the arthralgia patients that did and did not develop RA were not different, although the number of subjects was too low to allow within group comparisons.

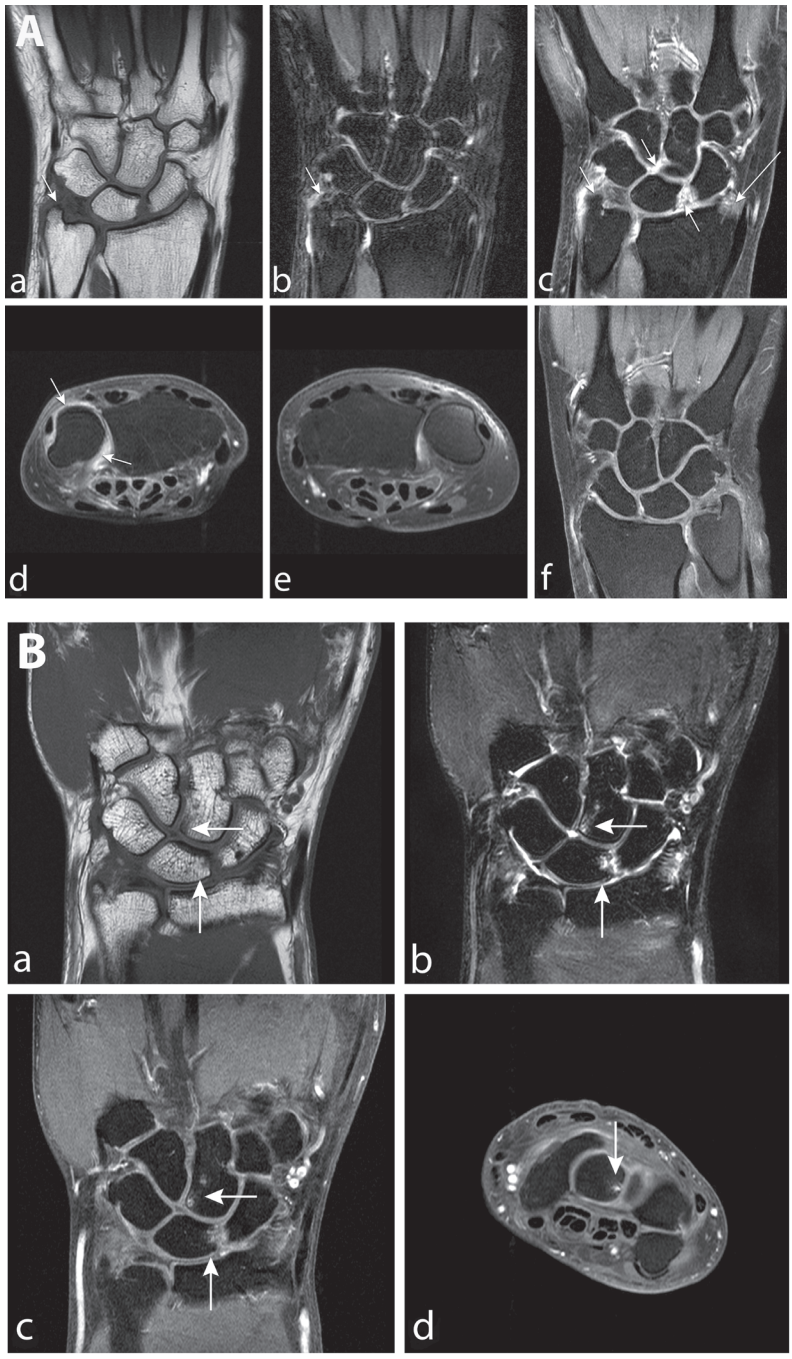
DISCUSSION

This study has explored whether subclinical inflammation occurs in painful small joints of ACPA positive arthralgia patients without clinical synovitis, using MRI to measure inflammation sensitively.[7] We observed differences between ACPA positive arthralgia patients, ACPA positive RA patients and symptom free controls. Furthermore, a correlation between local inflammation, as measured using MRI, and systemic inflammation, measured using CRP and ESR, was found in ACPA positive arthralgia patients. These data are in line with a study in monkeys showing that subclinical inflammation precedes clinical arthritis.[8] They are also in line with a recent ultra-sound study and a study reporting an increased signal on macrophage position emission tomography in ACPA positive arthralgia patients that developed clinical arthritis.[9,10] The present data are intriguing as they suggest that there is subclinical inflammation in ACPA positive patients in the preclinical phase. An example of synovitis and bone marrow oedema, as observed in ACPA positive arthralgia patients, is presented in figure 2.

Importantly, the present study used both positive and negative controls (RA patients and symptom free controls, respectively). In particular, the latter is crucial, as several studies have observed MRI abnormalities to a certain degree in healthy persons.[11-13]

This study has several limitations. First and most importantly, the numbers of subjects studied was small and the RAMRIS scores were quite low. The former is due to the low prevalence of ACPA positive arthralgia patients in outpatient clinics and the latter is due to measuring in very early disease stages. Consequently, the power to find statistically significant differences was limited. The power to perform within patient comparisons in particular was low. This was done when comparing within the group of ACPA positive arthralgia patients: MRI inflammation between painful and symptom free joints, level of local inflammation with systemic markers of inflammation (ESR and CRP) and MRI inflammation scores of patients that developed RA and those who did not develop RA. Larger studies are required to address these questions more extensively.

Another limitation is that the duration of follow-up was too short to definitely conclude which arthralgia patients progressed to RA. Also, in this respect, further studies with a longer follow-up of ACPA positive arthralgia patients are required.


A
a
b
c
d
e
f
B
a
b
c
d

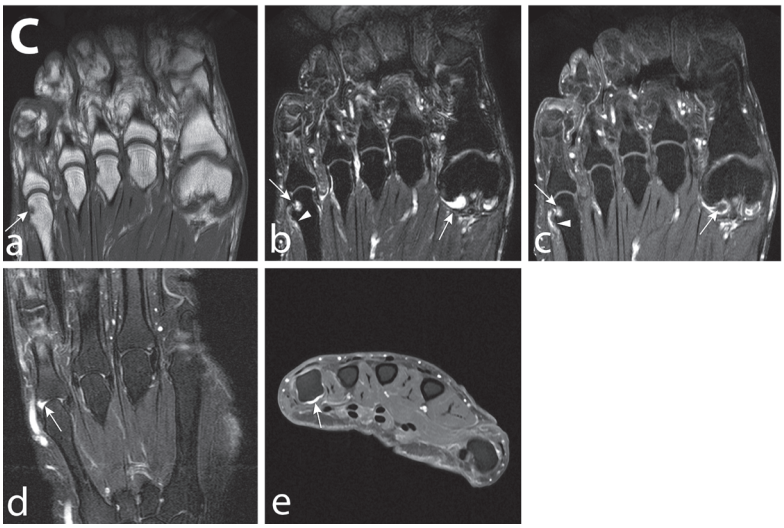


Figure 2 MR images of ACPA-positive arthralgia patients without clinically detectable arthritis.
(A) MR images of the left hand; the right hand is shown as comparison.
Coronal T1TSE, T2TSE fatsat and T1 fatsat after gadolinium (2Aa, b, c). Axial image at the level of the distal radioulnar joint (2Ad). The right hand is shown as comparison: T1 fatsat after gadolinium axial (2Ae) and coronal (2Af) without enhancement.
In the left hand; increased volume of soft tissues is appreciated around the distal ulna, intermediate of signal intensity on T1 (2Aa) high on T2 (2Ab) (arrow). After gadolinium abundant enhancement of thickened synovium is appreciated around the distal ulna and among the carpal joints as well (arrows 2Ac, d) According to the RAMRIS scoring method synovitis was scored. Partial volume is seen at the processus styloideus radii (long arrow).
(B) MR images of the left wrist
At the proximal end of the hamate and at the lunate regions of low intensity on the T1 (2Ba) and high intensity on T2 (2Bb) and high intensity on T1 after gadolinium (2Bc, d) are appreciated, which represents bone marrow edema. Furthermore, no enhancement of the synovium is seen (2Bc, d) Therefore, no synovitis was scored.
(C) MR images of the left foot (2Ca-c) and left hand (2Cd, e).
The head of metatarsal 5 showed a small lesion (arrows), low on T1 (2Ca), high on T2 (2Cb), enhancing after gadolinium (2Cc), which could be an erosion or synovial cyst. Subtle enhancement of synovium is seen laterally (arrowhead). A small amount of fluid with rim enhancement of synovium of MTP1 (long arrow) is present. No bone marrow edema is appreciated. The left hand shows a subtle amount of high signal intensity in MCP5, enhancing after gadolinium (2Cd, e), consistent with subtle synovitis.

A third limitation is that, for ethical reasons, the symptom free control group did not receive intravenous contrast, which may have underestimated synovitis scores in these people. However, differences were also observed in erosion and bone marrow oedema scores, evaluations which do not require contrast administration. Furthermore, the scanned joint regions were fixed in RA (MCP, wrist and MTP joints at the most painful side) and in the controls (MCP, wrist and MTP joints at the dominant site), but not in the ACPA positive

arthralgia patients. This was done in order to ensure that within ACPA positive arthralgia patients both joint regions with and without symptoms were evaluated. The number of painful joint regions in the arthralgia patients was low and a preset scanning protocol might have resulted in imaging of only symptom free joints.

In conclusion, the present study provides suggestive evidence that there is subclinical inflammation in ACPA positive arthralgia patients. Large longitudinal studies are needed to define the diagnostic or prognostic value of MRI in this preclinical phase.

REFERENCE LIST

1. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
2. Turesson C, Bergstrom U, Jacobsson LT, et al. Increased cartilage turnover and circulating autoantibodies in different subsets before the clinical onset of rheumatoid arthritis. *Ann Rheum Dis* 2011;70:520–2.
3. Chitale S, Ciapetti A, Hodgson R, et al. Magnetic resonance imaging and musculoskeletal ultrasonography detect and characterize covert inflammatory arthropathy in systemic sclerosis patients with arthralgia. *Rheumatology (Oxford)* 2010;49:2357–61.
4. van de Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. *Ann Rheum Dis* 2011;70:772–7.
5. Baan H, Bezooijen R, Avenarius JK, et al. Magnetic resonance imaging of the rheumatic foot according to the RAMRIS system is reliable. *J Rheumatol* 2011;38:1003–8.
6. Ostergaard M, Peterfy C, Conaghan P, et al. OMERACT Rheumatoid Arthritis Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. *J Rheumatol* 2003;30:1385–6.
7. Haavardsholm EA, Ostergaard M, Hammer HB, et al. Monitoring anti-TNFalpha treatment in rheumatoid arthritis: responsiveness of magnetic resonance imaging and ultrasonography of the dominant wrist joint compared with conventional measures of disease activity and structural damage. *Ann Rheum Dis* 2009;68:1572–9.
8. Kraan MC, Versendaal H, Jonker M, et al. Asymptomatic synovitis precedes clinically manifest arthritis. *Arthritis Rheum* 1998;41:1481–8.
9. Gent YY, Voskuyl AE, Kloet RW, et al. Macrophage positron emission tomography imaging as a biomarker for preclinical rheumatoid arthritis: findings of a prospective pilot study. *Arthritis Rheum* 2012;64:62–6.
10. van de Stadt LA, Bos WH, Meursinge RM, et al. The value of ultrasonography in predicting arthritis in auto-antibody positive arthralgia patients: a prospective cohort study. *Arthritis Res Ther* 2010;12:R98.
11. Ejbjerg B, Narvestad E, Rostrup E, et al. Magnetic resonance imaging of wrist and finger joints in healthy subjects occasionally shows changes resembling erosions and synovitis as seen in rheumatoid arthritis. *Arthritis Rheum* 2004;50:1097–106.
12. Olech E, Crues JV III, Yocum DE, et al. Bone marrow edema is the most specific finding for rheumatoid arthritis (RA) on noncontrast magnetic resonance imaging of the hands and wrists: a comparison of patients with RA and healthy controls. *J Rheumatol* 2010;37:265–74.
13. Palosaari K, Vuotila J, Soini I, et al. Small bone lesions resembling erosions can frequently be found in bilateral wrist MRI of healthy individuals. *Scand J Rheumatol* 2009;38:450–4.

Supplementary table 1 Overview of scanned joint regions.

	Scanned joint region		
	wrist	PIP/MCP	MTP
Symptom-free controls	19	19	19
ACPA+ arthralgia patients, symptom-free joint region	3	11	6
ACPA+ artralgia patients, painful joint region	3	8	10
ACPA+ RA patients	22	22	22

In ACPA-positive arthralgia-patients the most painful joint region was scanned. For comparison within patients; in these patients the symptom-free contralateral side was scanned as well. When both sides were painful, another non-painful region was scanned. In seven patients this resulted in another joint region scanned as symptom-free joint region. Two of them had painful MCP region and symptom-free MTP scanned joint region. In five patients the MTP joint region was scanned whereas MCP joint region was scanned as symptom-free joint region.

10

Concordance between inflammation at physical examination and on MRI in patients with early arthritis

A. Krabben, W. Stomp, T. W. J. Huizinga, D. van der Heijde, J. L. Bloem, M. Reijnierse, A. H. M. van der Helm-van Mil

Annals of the Rheumatic Diseases, 2013 December 12. Epub ahead of print.

ABSTRACT

Background

MRI is increasingly used to measure inflammation in rheumatoid arthritis (RA) research, but the correlation to clinical assessment is unexplored. This study determined the association and concordance between inflammation of small joints measured with MRI and physical examination.

Methods

179 patients with early arthritis underwent a 68 tender joint count and 66 swollen joint count and 1.5T MRI of MCP (2–5), wrist and MTP (1–5) joints at the most painful side. Two readers scored synovitis and bone marrow oedema (BME) according to the OMERACT RA MRI scoring method and assessed tenosynovitis. The MRI data were first analyzed continuously and then dichotomized to analyze the concordance with inflammation at joint examination.

Results

1790 joints of 179 patients were studied. Synovitis and tenosynovitis on MRI were independently associated with clinical swelling, in contrast to BME. In 86% of the swollen MCP joints and in 92% of the swollen wrist joints any inflammation on MRI was present. In 27% of the non-swollen MCP joints and in 66% of the non- swollen wrist joints any MRI inflammation was present. Vice versa, of all MCP, wrist and MTP joints with inflammation on MRI 64%, 61% and 77%, respectively, were not swollen. BME, also in case of severe lesions, occurred frequently in clinically non-swollen joints. Similar results were observed for joint tenderness.

Conclusions

Inflammation on MRI is not only present in clinically swollen but also in non-swollen joints. In particular BME occurred in clinically non-inflamed joints. The relevance of subclinical inflammation for the disease course is a subject for further studies.

INTRODUCTION

In recent years MRI is increasingly being used to measure disease states and treatment response in rheumatoid arthritis (RA) research.[1] The development of dedicated extremity-MRI and validated scoring methodology OMERACT RA MRI scoring (RAMRIS) has boosted the use of MRI. MRI has important advantages over conventional radiographs as, in addition to structural damage, inflammation of the synovium of the joints, tendons and bone (bone marrow oedema, BME) can be visualized and quantified.[2,3] BME is not detected by ultrasound or radiographs and is a strong predictor of erosive progression.[4-13] Recent data suggest that the presence of tenosynovitis in early arthritis is associated with progression to RA,[14] though more studies on this matter are required. Moreover, the exact role of MRI for diagnosing or prognostication of patients with early arthritis in clinical practice is not yet established.[15]

Independent of the purpose of MRI use, it is fundamental to understand to what extent abnormalities on MRI are concordant with abnormalities at physical examination of joints. A recent study found a moderate correlation between disease activity score in 28 joints and inflammation scores on MRI,[16] but the association between MRI and joint examination has never been studied thoroughly on joint level in patients with early arthritis. Given the general conception that MRI is more sensitive than physical examination; we assumed that inflammation detected at physical examination can be seen on MRI and that MRI may be able to detect more inflammatory lesions than physical examination. We aimed to address the following items: (i) To what extent joint swelling (or tenderness) at physical examination is associated with inflammation on MRI. (ii) In what proportion of swollen (or tender) joints is inflammation present on MRI. (iii) In what proportion of non-swollen (or non-tender) joints is inflammation present on MRI. (iv) Finally, we evaluated all joints that showed inflammation on MRI and determined how many of these joints are clinically not swollen (or non-tender). This last objective addressed the frequency of MRI inflammation that is undetected by clinical examination and therefore reflects subclinical inflammation.

PATIENTS AND METHODS

Patients

One hundred and seventy-nine patients with early arthritis were included in the Leiden Early Arthritis Clinic between August 2010 and February 2012 and underwent MRI at baseline. The Early Arthritis Clinic is a population-based inception cohort that includes patients with confirmed arthritis and symptoms for <2 years. At baseline, patients and rheumatologists completed questionnaires, a 68 tender joint count and 66 swollen joint count was performed by trained research nurses and serum was obtained.[17] Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee.

MRI scanning and scoring

The metacarpophalangeal (MCP) joints (second to fifth joints), wrist and (metatarsophalangeal) joints (first to fifth joints) at the most painful (or dominant side in case of equally severe symptoms at both sides) were scanned with an ONI-MSK-extreme 1.5T extremity-MRI-scanner (GE, Wisconsin, USA). For the MCP joints and wrists the following sequences were acquired: coronal T1 spin echo (SE) and T2 SE fatsat, and axial T1 SE fatsat post-gadolinium. For the MTP joints axial T1 SE and T2 SE fatsat sequences were obtained. Because of time limitations (the current protocol takes 75 min), post-gadolinium sequences were not obtained for the MTP joints. See the online supplementary methods for a detailed scan protocol. Synovitis and BME were quantitatively scored according to RAMRIS.[3] Tenosynovitis in the MCP joints and wrists was assessed as described by Haavardsholm et al.[2] The MRIs were scored by two readers independently, blinded to clinical data; the mean scores were studied. The within-reader intra class correlation coefficients for the total RAMRIS score were 0.98 and 0.83; the between-reader interclass correlation coefficient 0.82.

Analyses

Information on swelling of the MCP joints, wrists and MTP joints at physical examination of the scanned joints on the side that was MR-imaged was extracted from the joint count data. Joint tenderness is frequently considered as reflecting local inflammation and was also studied.

To make the MRI data comparable with the data on physical examination, the following items were summed. To calculate the BME score per MCP or MTP joint the score of the distal and the proximal bone was summed (range 0–6). Scoring the wrist according to the RAMRIS method implies that 15 bones are assessed, though not all of these are located around the radioulnar carpal joint that is assessed with physical examination. For the wrist we therefore evaluated the highest score of the bones lining the joint space: distally the proximal carpal row: scaphoid, lunate, triquetrum and pisiform and proximally the radius or ulna. These distal and proximal scores were summed (range 0–6).

The synovitis scores of the MCP and MTP joints were straightforward (range 0–3). The synovitis score of the wrist joint was the highest score of the radioulnar and radiocarpal compartment (range 0–3).

A swollen or tender joint at physical examination can also be (partly) due to tenosynovitis and therefore tenosynovitis was also assessed. To determine the tenosynovitis score per MCP joint we added up the scores of the extensor and the flexor tendons around the MCP joint (range 0–6). For the wrist, the highest score of the tenosynovitis around the extensors and around the flexors were summed (range 0–6). It was not possible to score tenosynovitis of the MTPs, because no coronal sequences, perpendicular to the axis of the metatarsals, were performed. See also figure 1 and online supplementary table S1 for an overview of score calculations.

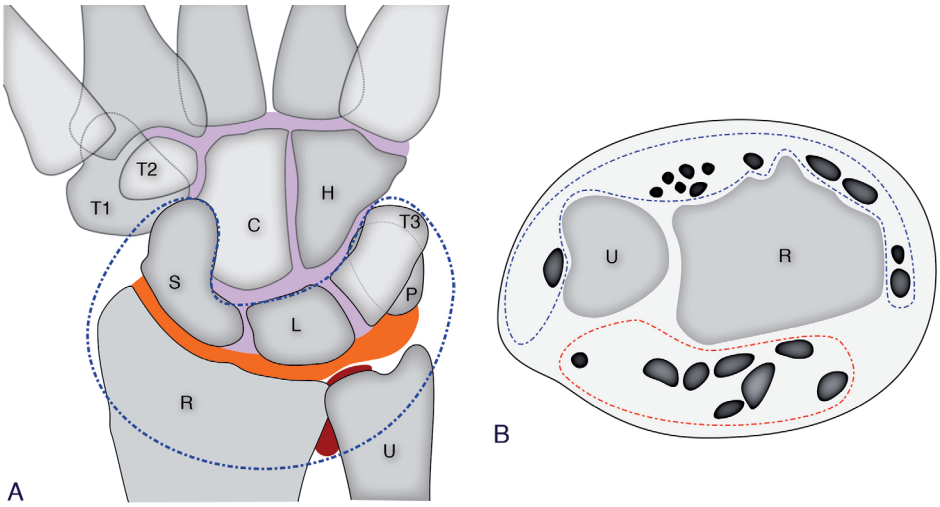


Figure 1 Overview of bones and joints in the wrist assessed for bone marrow oedema and synovitis (A) and the tendons assessed for tenosynovitis (B)
A) Coronal plane of the wrist, with the 5 metacarpal heads, trapezium (T1), trapezoid (T2), capitate (C), hamate (H), scaphoid (S), lunate (L), triquetrum (T3), pisiform (P), radius (R), ulna (U). The three synovial compartments are depicted in different colors: intercarpal (purple), radio-carpal (orange) and radio-ulnar (red).
To make the MRI score data comparable to the data at physical examination only the scores of the bones and synovial compartments within the dotted line were analysed. The bone marrow oedema score of the wrist was the sum of the distal and the proximal part. The distal bone marrow oedema score was the highest score among the scores of the scaphoid, lunate, triquetrum and pisiform. The proximal bone marrow oedema score was the highest score between the scores of the radius and the ulna. The synovitis score of the wrist was the highest score between the radio-carpal and the radio-ulnar compartment.
B) Axial plane of the wrist, with the radius (R) and the ulna (U) and all the tendons. To make one tenosynovitis score in the wrist we summed the flexor and the extensor score. The flexor score was the highest score among the tendons within the red dotted line. The extensor score was the highest score among the tendons within the blue dotted line.

First the association between inflammation detected by physical examination and MRI was studied per joint type, using logistic regression analyses with the continuous imaging scores as the independent variable and the joint examination results as dependent variable. Then, the association between inflammation detected by physical examination and by MRI was studied in all joints together. This analysis was done using generalized estimated equations as this model takes into account that in every patient 10 joints were assessed. Adjustments for age and gender were included. The exchangeable correlation matrix was used.

Subsequently the continuous MRI data were dichotomized to compare the concordance and discordance with physical examination. First, the cut-off of 1.0 was chosen; this score was achieved if the mean of both readers was ≥1.0. Two-by-two tables were made and

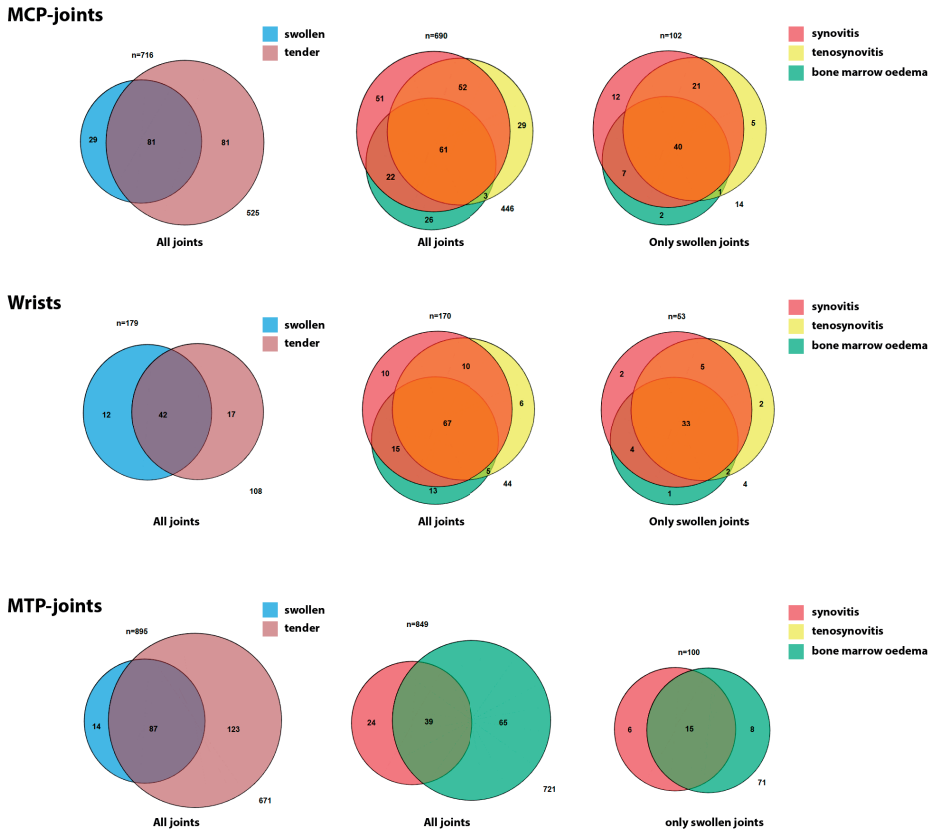


Figure 2 Inflammation features at physical examination and on MRI and their occurrences in MCP, wrist, MTP-joints.

proportions calculated. SPSS V.20.0 was used. p Values <0.05 were considered statistically significant.

RESULTS

Clinical baseline characteristics

Baseline characteristics of the 179 patients with early arthritis studied are presented in table 1. As shown in table 2, the percentage of patients with at least one clinically swollen or tender MCP, wrist and MTP joint ranged between 30% and 40%. Among the MCP and MTP joints, the second and the third joints were most frequently swollen and tender. The majority of swollen joints at examination (79%) were also tender. Conversely, 49% of the

Table 1 Characteristics of the 179 early arthritis patients

	(n=179)
Age, mean ± SD, years	54.1 ± 14.7
Female sex, n (%)	99 (55.3)
Symptom duration at inclusion, median (IQR), weeks	15 (6-27)
Onset of symptoms, n (%)	
(sub) acute	75 (43.4)
gradual	92 (51.4)
Morning stiffness, median (IQR), minutes	30 (15-60)
Swollen joint count, median (IQR)	3 (2-6)
Tender joint count, median (IQR)	6 (2-11)
ACPA positivity, n (%)	45 (25.1)
RF positivity, n (%)	54 (30.2)
CRP level, median (IQR)	4 (3-12)
ESR level, median (IQR)	14 (6-33)
Patient classification at baseline, n (%)	
RA (2010 ACR/EULAR criteria)	66 (37)
Unclassified arthritis	65 (36)
Psoriatric arthritis	15 (8)
Inflammatory osteoarthritis	12 (7)
Reactive arthritis	6 (3)
Other diagnoses	15 (8)

Interquartile range (IQR). Standard deviation (SD). 68 tender joint count and 66 swollen joint count were performed. At the two-weeks visit, when the results of laboratory and radiologic investigations were known, patients were classified according to the diagnoses presented.

Table 2 Frequency of patients with inflammation in MCP, wrist or MTP-joints at physical examination and MRI

		MCP-joints	Wrists	MTP-joints
Physical examination	≥ 1 swollen joint	36%	30%	25%
	≥ 1 tender joint	40%	11%	41%
MRI	Synovitis score ≥ 1	26%	60%	7%
	Tenosynovitis score ≥ 1	21%	52%	n.a.
	Bone marrow oedema score ≥ 1	17%	58%	12%

The frequencies at physical examination represent the frequencies of patients with at least 1 swollen or tender MCP, wrist or MTP-joint.
The frequencies at MR imaging represent the frequencies of all MCP, wrist or MTP-joints that had a synovitis, tenosynovitis or BME score of at least 1.
n.a.= not assessed
For technical reasons some joints on MRI could not be evaluated. Of all 716 MCP-joints; 8 synovitis, 17 tenosynovitis and 14 BME scores were not assessed. Of all 179 wrists; 1 synovitis, 2 tenosynovitis and 6 BME scores were not assessed. Of all 895 MTP-joints; 29 synovitis and 45 BME scores were not assessed.

tender joints were also swollen at physical examination. The relation per joint region is depicted in figure 2.

MRI baseline characteristics

Per patient 10 joints (4 MCPs, 1 wrist and 5 MTPs) were scanned. The frequencies of synovitis, tenosynovitis and BME are presented in table 2. In the wrists these features were more frequently present than in the MCP and MTP joints. Among the MCP joints, the second and the third MCPs were most frequently affected by synovitis, tenosynovitis and BME. In the wrist, the lunate showed most frequently BME (41% had a score ≥1) and among the MTP joints synovitis, tenosynovitis and BME were most often present in MTP1. The MRI features of inflammation frequently occurred together (figure 2).

Association between inflammation detected by MRI and physical examination

First the association between inflammation on MRI and at physical examination was analyzed per joint type (eg, all MCP2 joints). The OR on joint swelling for every unit increase in MRI score is depicted in figure 3. All three inflammatory measures visualized on MRI were significantly associated with the presence of joint swelling at physical examination. Among

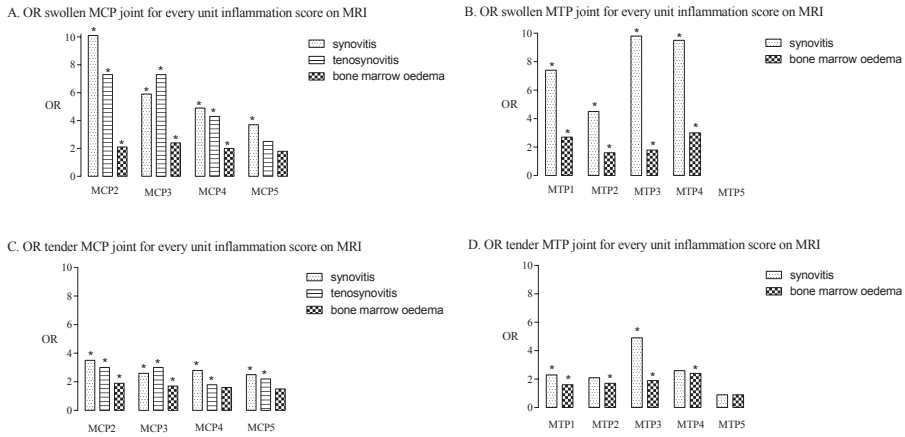


Figure 3 The association between inflammation detected by MRI and physical examination, for every MCP and MTP-joint separately.
*=p<0.05. The association between inflammation detected by physical examination and MRI was studied per joint type, using logistic regression analyses with the continuous imaging scores as independent variable and the joint examination result as dependent variable. The Odds Ratio (OR) reflects the ratio between the odds on joint swelling and the odds on no joint swelling for every unit increase in MRI score per joint type. It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

the MCP joints the strongest associations were observed for MCP2 and MCP3 joints and among the MTP joints for MTP3 and MTP4 joints.

Second for all 1790 joints together the association between inflammation detected by MRI and physical examination was analyzed. All three inflammatory measures visualized on MRI were significantly associated with the presence of joint swelling at physical examination. Synovitis on MRI was more strongly associated with clinical joint swelling than with tenosynovitis or BME. As the MRI inflammation features often occurred together, all three features were entered in one analysis, to evaluate which of these variables were independently associated with clinical joint swelling. In the MCP joints, synovitis and tenosynovitis were independently associated with clinical swelling (OR 3.3 and 2.4, $p<0.001$), but BME was not independently associated with joint swelling (OR 1.2, $p=0.13$). In the wrist, similar results were obtained: synovitis and tenosynovitis were independently associated to clinical swelling (OR 2.3 and 1.8, $p=0.03$), in contrast to BME (OR 1.0, $p=0.8$) (see online supplementary table S2).

Frequency of MRI inflammation in clinically swollen joints

Subsequently the continuous MRI scoring data were dichotomized and the concordance and discordance between inflammation detected by physical examination and MRI studied (table 3A). Of all swollen MCP joints at physical examination, any MRI measure of inflammation was present in 86%, synovitis in 73%, tenosynovitis in 65% and BME in 50%. Of all swollen wrists, any MRI measure of inflammation was present in 92%, synovitis in 83%, tenosynovitis in 78% and BME in 75%. Of all swollen MTP joints, synovitis was seen in 21%, BME in 23% and any of these features in 29%.

Frequency of MRI inflammation in clinically non-swollen joints

Of all non-swollen MCP joints at physical examination, any MRI measure of inflammation was observed in 27%, synovitis in 18%, tenosynovitis in 13% and BME in 10%. Of all non-swollen wrists, any MRI measure of inflammation was observed in 66%, synovitis in 50%, tenosynovitis in 41% and BME in 51%. Of all non-swollen MTP joints, any MRI measure of inflammation was observed in 13%, synovitis in 5% and BME in 11% (table 3A).

Frequency of clinically non-swollen joints in the presence of inflammation on MRI

Subsequently, it was studied how often physical joint examination was normal, given the presence of inflammation on MRI, indicating what proportion of MRI abnormalities is clinically not detected.

First all MCP joints that revealed any inflammation on MRI were evaluated. Of these, 64% were not swollen at physical examination. Then the MRI inflammatory features were studied separately. Of all MCP joints that had synovitis, 57% were not swollen. Of all MCP

Table 3A Inflammation on MRI in relation to joint swelling at physical examination.

	MCP-joints								
	Synovitis score			Tenosynovitis score			Bone marrow oedema score		
(n=)	≥1 *(187)*	≥2 *(40)*	≥3 *(2)*	≥1 *(145)*	≥2 *(34)*	≥3 *(2)*	≥1 *(116)*	≥2 *(43)*	≥3 *(23)*
% of swollen joints with MRI feature	73%	25%	2%#	65%	18%	2%#	50%	23%	13%
% of *not* swollen joints with MRI feature	18%	2%	0%#	13%	3%	0%#	10%	3%	2%
Given MRI inflammation, % of non-swollen joints	57%	32%	0%#	54%	44%	0%#	53%	42%	39%
	Wrists								
	Synovitis score			Tenosynovitis score			Bone marrow oedema score		
(n=)	≥1 *(107)*	≥2 *(39)*	≥3 *(2)*	≥1 *(92)*	≥2 *(39)*	≥3 *(6)*	≥1 *(101)*	≥2 *(53)*	≥3 *(26)*
% of swollen joints with MRI feature	83%	48%	4%#	78%	48%	7%#	75%	55%	32%
% of *not* swollen joints with MRI feature	50%	10%	0%#	41%	11%	2%#	51%	20%	7%
Given MRI inflammation, % of non-swollen joints	58%	33%	0%#	54%	33%	33%#	60%	45%	35%
	MTP-joints†								
	Synovitis score						Bone marrow oedema score		
(n=)	≥1 *(63)*	≥2 *(4)*	≥3 *(0)*				≥1 *(105)*	≥2 *(49)*	≥3 *(31)*
% of swollen joints with MRI feature	21%	1%#	n.ap.				23%	17%	13%
% of *not* swollen joints with MRI feature	5%	0%#	n.ap.				11%	4%	2%
Given MRI inflammation, % of non-swollen joints	67%	75%#	n.ap.				78%	65%	58%

n.ap.= not applicable because n=0.

† It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

these frequencies should be interpreted with discretion, because of the low numbers of joints with these scores.

Table 3B Inflammation on MRI in relation to tenderness at physical examination.

	MCP-joints								
	Synovitis score			Tenosynovitis score			Bone marrow oedema score		
(n=)	≥1 *(187)*	≥2 *(40)*	≥3 *(2)*	≥1 *(145)*	≥2 *(34)*	≥3 *(2)*	≥1 *(116)*	≥2 *(43)*	≥3 *(23)*
% of tender joints with MRI feature	51%	15%	1%#	42%	9%	1%#	36%	15%	8%
% of *not* tender joints with MRI feature	19%	3%	0%#	15%	4%	0%#	11%	4%	2%
Given MRI inflammation, % of non-tender joints	56%	40%	0%#	54%	56%	0%#	49%	44%	43%
	Wrists								
	Synovitis score			Tenosynovitis score			Bone marrow oedema score		
(n=)	≥1 *(107)*	≥2 *(39)*	≥3 *(2)*	≥1 *(92)*	≥2 *(39)*	≥3 *(6)*	≥1 *(101)*	≥2 *(53)*	≥3 *(26)*
% of tender joints with MRI feature	88%	42%	2%#	83%	41%	5%#	76%	59%	31%
% of *not* tender joints with MRI feature	46%	22%	1%#	37%	13%	3%#	50%	17%	7%
Given MRI inflammation, % of non-tender joints	51%	46%	50%#	48%	38%	50%#	56%	36%	31%
	MTP-joints †								
	Synovitis score						Bone marrow oedema score		
(n=)	≥1 *(63)*	≥2 *(4)*	≥3 *(0)*				≥1 *(105)*	≥2 *(49)*	≥3 *(31)*
% of tender joints with MRI feature	13%	1%	n.ap.				20%	13%	9%
% of *not* tender joints with MRI feature	6%	0%	n.ap.				10%	4%	2%
Given MRI inflammation, % of non-tender joints	59%	50%	n.ap.				62%	49%	45%

n.ap.= not applicable because n=0.

† It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

these frequencies should be interpreted with discretion, because of the low numbers of joints with these scores.

joints with tenosynovitis, 54% were not swollen. Of all MCP joints with BME this was 53%. Thereafter, these frequencies were determined for more severe inflammatory features on MRI (cut-off scores ≥2 and ≥3). The frequencies of MCP joints with synovitis, tenosynovitis or BME on MRI that were clinically not swollen, decreased (table 3A).

Next, the wrists that showed inflammation on MRI were evaluated in the same way. Of these joints with any inflammation on MRI, 61% were not swollen at physical examination. Of all wrists that had synovitis, tenosynovitis or BME, 58%, 54% and 60%, respectively, were not swollen. When using higher cut-offs to define MRI inflammation (scores ≥2 and ≥3), the part of subclinical synovitis (synovitis at MRI but not at physical examination) of all detected synovitis decreased. However, in the wrists with tenosynovitis score ≥3 and BME score ≥3, 33% and 35% were still clinically not swollen.

When evaluating the MTP joints that showed any inflammation on MRI, 77% were not swollen at physical examination. With regards to synovitis, the majority of MTP joints had a score of ≥1; 67% of these joints were not swollen at physical examination. For BME, 78% of the MTP joints that had BME (score ≥1), were not swollen, indicating the presence of subclinical inflammation. Also when assessing the MTP joints with high BME scores (score ≥3), 58% were clinically not swollen. Hence, the proportion of subclinical BME of all detected BME, was the highest in the feet (table 3A).

Analysis on MRI inflammation in relation to joint tenderness at physical examination

Since joint tenderness at physical examination can be considered as a sign of inflammation, all analyses mentioned above were repeated with joint tenderness instead of joint swelling. The association between inflammation on MRI and joint tenderness was less strong (lower OR) than the association between joint swelling (see online supplementary table S2). The concordance between inflammation on MRI and joint tenderness was almost similar (table 3B), but the frequency of MRI inflammation in tender MCP joints was less than in swollen MCP joints. Also here, when evaluating all joints with any inflammation on MRI, a large proportion was not tender at examination (56% for MCP joints, 51% for wrists and 59% for MTP joints). Additionally, it was observed that of the joints with relative severe BME (score ≥3), 31–45% were still not tender (table 4) and 19–45% were neither swollen nor tender. Also here these frequencies were the highest in the feet.

Sub-analyses in early RA

Subsequently we questioned whether the results were different when evaluating only the subgroup of patients with early arthritis diagnosed with RA (2010 criteria; n=66) at first visit. Here the prevalence of all MRI inflammation features was higher than in the total group of patients with early arthritis. The concordance analyses were repeated; overall this did not yield different conclusions (see online supplementary table S3 A,B).

Serological markers and subclinical inflammation

Additionally, the prevalence of subclinical inflammation in patients positive and patients negative for serological markers was evaluated. We observed that BME (score ≥1) was more prevalent in non-swollen joints of anti-citrullinated peptide antibody (ACPA) positive patients than in that of ACPA negative patients (p=0.01). Synovitis and tenosynovitis were not significantly different (p=0.65 and p=0.56, respectively). Similar findings were found for rheumatoid factor (RF) RF positive and negative patients (p=0.047, 0.82, 0.17; for BME, synovitis and tenosynovitis). When comparing MRI inflammation in non-swollen joints of patients with and without a raised C reactive protein (CRP), no different prevalence was observed (p=0.82, 0.46 and 0.19, respectively).

DISCUSSION

This study investigated whether joint swelling, which is conceived as a reflection of local inflammation at physical examination, is associated with inflammation reflected by MRI. This study of patients with early arthritis showed that inflammation on MRI is present in the large majority of clinically swollen joints and in non-swollen joints. Furthermore, a high proportion of joints with MRI inflammation had no signs of clinical inflammation. For instance; 35–58% of the joints with severe BME, a MRI feature that was associated with erosive progression in other studies, were non-swollen at physical examination.

All three features of inflammation depicted by MRI (synovitis, tenosynovitis and BME) were significantly associated with the presence of clinical joint swelling. As expected, synovitis had the strongest association. Furthermore, the MRI features of inflammation frequently occurred together in the same joint and the association between BME and clinical swelling was not independent of synovitis and tenosynovitis.

The highest frequencies of MRI inflammation scores were seen in the wrists. More bones, synovium compartments and tendons were assessed in wrists than in MCP and MTP joints. With regards to BME in the wrist, we decided not to evaluate all carpal bones included in the RAMRIS but only the bones that are located around the joint space. This was done to have the same anatomical area investigated by physical examination and MRI. Nonetheless, the percentage of joints, swollen and non-swollen, with inflammatory features on MRI was higher in wrists than in MCP and MTP joints.

Limitations of the present study are the absence of coronal sequences, perpendicular to the axis of the metatarsals, making it impossible to evaluate tenosynovitis of the forefoot. The absence of post-contrast images of the foot resulted in less reliable scoring of synovitis, because hyperplasic inflamed synovium could not be adequately differentiated from fluid in the MTP joints. The absence of post-contrast images may hypothetically have resulted in either overestimation or underestimation of MRI synovitis and therefore the present data

do not allow drawing definitive conclusions on the association between synovitis detected by MRI and physical examination of the foot.

For BME evaluation however, contrast enhancement is not required. Interestingly, of all joints with BME score ≥1, the majority was not clinically inflamed and this proportion was the highest in the feet. This indicates that with respect to BME, subclinical inflammation is most often present in the feet. The fact that MTP joints are more difficult to physically examine than other small joints may play a role here.

Hence, we observed that BME is not independently associated with joint swelling and that joints with BME are frequently not swollen (in case of joints with a BME score ≥1 this concerned 53–78% and in joints with a BME score ≥3 this was 35–58%). BME is previously observed to be a prognosticator for erosive joint damage progression in RA.[6-8,10,18] In patients with unclassified arthritis, BME was predictive for progression to RA.[19] BME is also called osteitis, as studies that combined MRI and histology observed inflammatory infiltrates at the location of BME. In these samples; osteoclasts were observed nearby, suggesting a link between inflammation and erosive bone damage.[20] A previous study showed that clinically relevant progression of joint damage does occur in patients with RA in prolonged remission.[21] We showed that BME is also present in the non-swollen joints and by knowing that BME is predictive for erosive progression, BME could reflect subclinical inflammation predicting progression during periods of low disease activity. Unfortunately, in this study we had no longitudinal data and therefore we could not draw conclusions about the follow-up of the non-swollen joints with BME.

Because a joint can be clinically swollen due to inflammation of the synovium of the joints and the tendons, tenosynovitis was scored in addition to synovitis. Both features indeed presented frequently in the same joint. When tenosynovitis was present, synovitis occurred in 78–88%. As far as we know this finding is not reported in previous studies and more research on the value of tenosynovitis as part of joint inflammation in early arthritis is needed.

The evaluation of synovitis and tenosynovitis of the MCP joints and wrists was not hampered by technical issues and we observed that synovitis and tenosynovitis occurred in clinically non-swollen joints. The frequency of this subclinical inflammation depended on the degree of MRI inflammation. For instance, when MRI-synovitis was present with a score ≥1, 57–58% of the joints were not swollen and in case of joints with a MRI synovitis score ≥2 this was 32–33%. One explanation of this finding is that MRI is more sensitive for identifying synovitis than physical joint examination. Alternatively, there are many false-positive findings if we rely on minimal findings.

Joint swelling was studied as the main parameter for clinical joint inflammation. Joint tenderness was assessed as well. The association of inflammation on MRI with swollen joints was stronger than with tender joints. This can possibly be explained by the tender joint count being more frequently affected by processes other than inflammation.

In conclusion, this study observed a high frequency of subclinical inflammation in patients with early arthritis, detected by MRI. The relevance of subclinical inflammation for the disease course is a subject for further studies, though previous studies have indicated that BME is particularly associated with disease progression.

REFERENCE LIST

1. van der Helm-van Mil AH. Imaging: Use of MRI as an outcome measure in clinical trials in RA. *Nat Rev Rheumatol* 2012;8:643–4.
2. Haavardsholm EA, Ostergaard M, Ejbjerg BJ, et al. Introduction of a novel magnetic resonance imaging tenosynovitis score for rheumatoid arthritis: reliability in a multireader longitudinal study. *Ann Rheum Dis* 2007;66:1216–20.
3. Ostergaard M, Edmonds J, McQueen F, et al. An introduction to the EULAR-OMERACT rheumatoid arthritis MRI reference image atlas. *Ann Rheum Dis* 2005;64(Suppl 1):i3–7.
4. Boyesen P, Haavardsholm EA, van der Heijde D, et al. Prediction of MRI erosive progression: a comparison of modern imaging modalities in early rheumatoid arthritis patients. *Ann Rheum Dis* 2011;70:176–9.
5. Dohn UM, Ejbjerg B, Boonen A, et al. No overall progression and occasional repair of erosions despite persistent inflammation in adalimumab-treated rheumatoid arthritis patients: results from a longitudinal comparative MRI, ultrasonography, CT and radiography study. *Ann Rheum Dis* 2011;70:252–8.
6. Haavardsholm EA, Boyesen P, Ostergaard M, et al. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. *Ann Rheum Dis* 2008;67:794–800.
7. Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009;68:384–90.
8. Hetland ML, Stengaard-Pedersen K, Junker P, et al. Radiographic progression and remission rates in early rheumatoid arthritis—MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 2010;69:1789–95.
9. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 1999;58:156–63.
10. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003;48:1814–27.
11. Mundwiler ML, Maranian P, Brown DH, et al. The utility of MRI in predicting radiographic erosions in the metatarsophalangeal joints of the rheumatoid foot: a prospective longitudinal cohort study. *Arthritis Res Ther* 2009;11:R94.
12. Palosaari K, Vuotila J, Takalo R, et al. Bone oedema predicts erosive progression on wrist MRI in early RA—a 2-yr observational MRI and NC scintigraphy study. *Rheumatology (Oxford)* 2006;45:1542–8.
13. Savnik A, Bliddal H, Nyengaard JR, et al. MRI of the arthritic finger joints: synovial membrane volume determination, a manual vs a stereologic method. *Eur Radiol* 2002;12:94–8.
14. Navalho M, Resende C, Rodrigues AM, et al. Bilateral MR imaging of the hand and wrist in early and very early inflammatory arthritis: tenosynovitis is associated with progression to rheumatoid arthritis. *Radiology* 2012;264:823–33.
15. Machado PM, Koevoets R, Bombardier C, et al. The value of magnetic resonance imaging and ultrasound in undifferentiated arthritis: a systematic review. *J Rheumatol* Suppl 2011;87:31–7.
16. Emery P, van der Heijde D, Ostergaard M, et al. Exploratory analyses of the association of MRI with clinical, laboratory and radiographic findings in patients with rheumatoid arthritis. *Ann Rheum Dis* 2011;70:2126–30.
17. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes— what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011;50:93–100.

18. Boyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011;70:428–33.
19. Duer-Jensen A, Horslev-Petersen K, Hetland ML, et al. Bone edema on magnetic resonance imaging is an independent predictor of rheumatoid arthritis development in patients with early undifferentiated arthritis. *Arthritis Rheum* 2011;63:2192–202.
20. Dalbeth N, Smith T, Gray S, et al. Cellular characterisation of magnetic resonance imaging bone oedema in rheumatoid arthritis; implications for pathogenesis of erosive disease. *Ann Rheum Dis* 2009;68:279–82.
21. Molenaar ET, Voskuyl AE, Dinant HJ, et al. Progression of radiologic damage in patients with rheumatoid arthritis in clinical remission. *Arthritis Rheum* 2004;50:36–42.

Supplementary Table 1 Overview joint score calculations

	Synovitis (0-3)	Tenosynovitis (0-6)	Bone marrow oedema (range:0-6)
MCP-joints	MCP5	Flexor MCP5+extensor MCP5	MCP5distal+MCP5proximal
	MCP4	Flexor MCP4+extensor MCP4	MCP4distal+MCP4proximal
	MCP3	Flexor MCP3+extensor MCP3	MCP3distal+MCP3proximal
	MCP2	Flexor MCP2+extensor MCP2	MCP2distal+MCP2proximal
wrists	Highest score of: radio-ulnar, radio-carpal	Highest score of: I, II, III, IV, V, VI (extensors) + Highest score of: 1, 2, 3, 4 (flexors)	Highest score of: pisiform, triquetrum, lunate, scaphoid (distal) + Highest score of: ulna, radius (proximal)
MTP-joints	MTP5	n.a.	MTP5distal+MTP5proximal
	MTP4		MTP4distal+MTP4proximal
	MTP3		MTP3distal+MTP3proximal
	MTP2		MTP2distal+MTP2proximal
	MTP1		MTP1distal+MTP1proximal

n.a.= not assessed. It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

Supplementary Table 2 Association between inflammation detected by MRI and physical examination.

	Association with clinically swollen joint					
	MCP-joints		Wrists		MTP-joints	
	OR	p	OR	p	OR	p
Synovitis	5.9	<0.001	4.0	<0.001	2.0	0.02
Tenosynovitis	5.5	<0.001	2.7	<0.001	n.a.	n.a.
Bone marrow oedema	2.3	<0.001	1.7	<0.001	1.4	0.01
All three together in one model:						
Synovitis	3.3	<0.001	2.3	0.03	1.1	0.74
Tenosynovitis	2.4	<0.001	1.8	0.03	n.a.	n.a.
Bone marrow oedema	1.2	0.13	1.0	0.80	1.3	0.04
	Association with clinically tender joint					
	MCP-joints		Wrists		MTP-joints	
	OR	p	OR	p	OR	p
Synovitis	2.9	<0.001	3.5	<0.001	1.7	0.01
Tenosynovitis	2.5	<0.001	2.4	<0.001	n.a	n.a.
Bone marrow oedema	1.8	<0.001	1.7	0.002	1.3	0.003
All three together in one model:						
Synovitis	1.9	0.001	2.0	0.09	1.1	0.65
Tenosynovitis	1.5	0.03	1.6	0.07	n.a.	n.a.
Bone marrow oedema	1.3	0.03	1.1	0.55	1.3	0.04

All analyses were adjusted for age and gender.
n.a.= not assessed. It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.
The association between inflammation detected by physical examination and by MRI was studied in all joints together. This analysis was done using Generalized Estimated Equations as this model takes into account that in every patient 10 joints were assessed. Adjustments for age and gender were included. The Odds Ratio (OR) reflects the ratio between the odds on joint swelling and the odds on no joint swelling for every unit increase in MRI score. Depicted are the ORs of the three MRI inflammation features tested separately and tested together in one analysis.

Supplementary Table 3A Subanalyses in RA patients (n=66): Inflammation on MRI in relation to swelling at physical examination.

	MCP-joints								
	Synovitis score			Tenosynovitis score			Bone marrow edema score		
(n=)	≥1 *(79)*	≥2 *(16)*	≥3 *(1)*	≥1 *(69)*	≥2 *(19)*	≥3 *(2)*	≥1 *(53)*	≥2 *(19)*	≥3 *(9)*
% of swollen joints with MRI feature	73%	24%	2%#	65%	19%	4%#	45%	25%	11%#
% of *not* swollen joints with MRI feature	19%	1%	0%#	17%	4%	0%#	14%	3%	1%#
Given MRI inflammation, % of non-swollen joints	49%	19%	0%#	51%	47%	0%#	55%	32%	33%#
	Wrists								
	Synovitis score			Tenosynovitis score			Bone marrow edema score		
(n=)	≥1 *(42)*	≥2 *(19)*	≥3 *(1)*	≥1 *(39)*	≥2 *(20)*	≥3 *(3)*	≥1 *(43)*	≥2 *(22)*	≥3 *(12)*
% of swollen joints with MRI feature	90%	60%	5%#	80%	60%	10%#	79%	63%	37%
% of *not* swollen joints with MRI feature	53%	16%	0%#	51%	18%	2%#	64%	23%	11%
Given MRI inflammation, % of non-swollen joints	57%	37%	0%#	59%	40%	33%#	65%	45%	42%
	MTP-joints †								
	Synovitis score						Bone marrow edema score		
(n=)	≥1 *(31)*	≥2 *(2)*	≥3 *(0)*				≥1 *(52)*	≥2 *(28)*	≥3 *(16)*
% of swollen joints with MRI feature	23%	0%#	n.ap.				21%	12%	11%
% of *not* swollen joints with MRI feature	7%	1%#	n.ap.				15%	8%	4%
Given MRI inflammation, % of non-swollen joints	58%	100%#	n.ap.				77%	75%	63%

n.ap.= not applicable because n=0.

† It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

these frequencies should be interpreted with discretion, because of the low numbers of joints with these scores.

Supplementary Table 3B Subanalyses in RA patients (n=66): Inflammation on MRI in relation to tenderness at physical examination.

	MCP-joints								
	Synovitis score			Tenosynovitis score			Bone marrow edema score		
(n=)	≥1 *(79)*	≥2 *(16)*	≥3 *(1)*	≥1 *(69)*	≥2 *(19)*	≥3 *(2)*	≥1 *(53)*	≥2 *(19)*	≥3 *(9)*
% of tender joints with MRI feature	52%	14%	1%#	42%	9%	3%#	32%	14%	6%#
% of *not* tender joints with MRI feature	21%	3%	0%#	20%	7%	0%#	16%	4%	2%#
Given MRI inflammation, % of non-tender joints	49%	31%	0%#	54%	63%	0%#	53%	42%	44%#
	Wrists								
	Synovitis score			Tenosynovitis score			Bone marrow edema score		
(n=)	≥1 *(42)*	≥2 *(19)*	≥3 *(1)*	≥1 *(39)*	≥2 *(20)*	≥3 *(3)*	≥1 *(43)*	≥2 *(22)*	≥3 *(12)*
% of tender joints with MRI feature	96%	57%	4%#	87%	43%	4%#	86%	68%	36%
% of *not* tender joints with MRI feature	48%	14%	0%#	45%	24%	5%#	59%	17%	10%
Given MRI inflammation, % of non-tender joints	48%	32%	0%#	49%	50%	67%#	56%	32%	33%
	MTP-joints †								
	Synovitis score						Bone marrow edema score		
(n=)	≥1 *(31)*	≥2 *(2)*	≥3 *(0)*				≥1 *(52)*	≥2 *(28)*	≥3 *(16)*
% of tender joints with MRI feature	15%	1%#	n.ap.				22%	13%	8%
% of *not* tender joints with MRI feature	7%	0%#	n.ap.				13%	6%	3%
Given MRI inflammation, % of non-tender joints	45%	50%#	n.ap.				52%	46%	44%

n.ap.= not applicable because n=0.

† It was not possible to score tenosynovitis of the MTPs, because here no MR-images right-angled (coronal planes) were available.

these frequencies should be interpreted with discretion, because of the very low numbers.

SUPPLEMENTARY METHODS: MRI SCAN PROTOCOL

MR imaging of the hand (wrist and metacarpophalangeal joints) and forefoot (metatarsophalangeal joints) was performed within two weeks after inclusion, at the most painful side, or in case of completely symmetric symptoms at the dominant side. The presence of clinical arthritis at physical examination of the joints that were scanned was not a prerequisite. Two patients were excluded because of contraindications for MR imaging. Patients with impaired renal function or known hypersensitivity or allergic reactions to contrast media were imaged without contrast administration (n=2).

MR imaging was performed on a MSK-extreme 1.5T extremity MR imaging system (GE, Wisconsin, USA) using a 145mm coil for the foot and a 100mm coil for the hand. The patient was positioned in a chair beside the scanner, with the hand or foot fixed in the coil with cushions.

The forefoot was scanned using a T1-weighted fast spin-echo (FSE) sequence in the axial plane with repetition time (TR) of 650 ms, echo time (TE) 17ms, acquisition matrix, 388×288, echo train length (ETL) 2; and a T2-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 3000/61.8; acquisition matrix 300x224, ETL7). Due to time constraints, imaging of the foot was limited to pre-contrast sequences only.

In the hand, the following sequences were acquired before contrast injection: T1-weighted FSE sequence in the coronal plane (TR/TE 650/17ms; acquisition matrix 388×88; ETL2); T2-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 3000/61.8ms; acquisition matrix, 300x224, ETL7). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 650/17ms, acquisition matrix 364×224, ETL2), T1-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 570/7 ms; acquisition matrix 320x192; ETL2).

Field-of-view was 100mm for the hand and 140mm for the foot. Coronal sequences had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. All axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm, with 20 slices for the hand and 16 for the foot. Total imaging time was approximately 75 minutes.

MRI detected subclinical joint inflammation is associated with radiographic progression

A. Krabben, W. Stomp, J. A. B. van Nies, T. W. J. Huizinga,
D. van der Heijde, J. L. Bloem, M. Reijnierse,
A. H. M. van der Helm-van Mil

Annals of the Rheumatic Diseases, 2014 July 29. Epub ahead of print.

ABSTRACT

Background

We recently demonstrated that MRI inflammation is prevalent in clinically non-swollen joints of early arthritis patients. In this study we assessed the relevance of this subclinical inflammation with regard to radiographic progression.

Methods

1,130 joints (unilateral MCP2-5, wrist, and MTP1-5) of 113 early arthritis patients underwent clinical examination and 1.5T MRI at baseline and radiographs at baseline and 1-year. Two readers scored the MRIs for synovitis, bone marrow edema (BME) and tenosynovitis according to RAMRIS. Radiographic progression over 1-year was determined using the Sharp-van-der-Heijde-scoring method.

Results

On patient level; BME, synovitis and tenosynovitis were associated with radiographic progression, independent of known risk factors (p=0.003, 0.001 and 0.011, respectively). Of all non-swollen joints (n=932), 232 joints (26%) had subclinical inflammation (≥1 MRI-inflammation feature present). These joints were distributed among 91% of patients. Radiographic progression was present in 4% of non-swollen joints with subclinical inflammation compared to 1% of non-swollen joints without subclinical inflammation (RR3.5, 95%CI 1.3-9.6). Similar observations were done for BME (RR5.3, 95%CI 2.0-14.0), synovitis (RR3.4, 95%CI 1.2-9.3) and tenosynovitis (RR3.0, 95%CI 0.7-12.7) separately.

Conclusions

Radiographic progression was infrequent but joints with subclinical inflammation had an increased risk of radiographic progression within year-1. This demonstrates the relevance of MRI-detected subclinical inflammation.

INTRODUCTION

The severity of radiographic joint destruction varies between RA-patients. Much research has been focused on identifying risk factors for radiographic progression, with the ultimate aim of achieving individualized medicine at disease onset. Well-known risk factors are presence of erosions, presence of RA-related auto-antibodies (rheumatoid factor (RF), anti-citrullinated-peptide antibodies (ACPA)) and measures of inflammation (C-reactive protein (CRP), number of swollen joints (SJC)).

MRI measures inflammation sensitively; it allows assessment of bone marrow edema (BME), tenosynovitis and synovitis. Previous studies in wrist and MCP-joints showed that inflammation detected with MRI, especially BME, is a strong predictor for radiographic progression (Suppl Table 1).[1-8] However, none of these studies investigated MRI findings in relation to clinical joint inflammation. We recently studied MRI-inflammation in small joints that were not swollen at physical examination and observed MRI-inflammation in 27%, 66% and 13% of non-swollen MCP, wrist and MTP-joints of early arthritis patients.[9] The identification of these inflamed joints may indicate the added value of MRI. However, the relevance of such subclinical inflammation is supported when it associates with radiographic progression. In this study we therefore investigated whether MRI-detected subclinical inflammation is associated with radiographic progression.

PATIENTS AND METHODS

Patients

Between August 2010 and February 2012, MRI was performed in 179 early arthritis patients of the Leiden Early Arthritis Clinic, a population-based inception-cohort including patients with confirmed clinical arthritis and symptoms for <2 years. At baseline, questionnaires and 66-SJCs were performed and serum obtained.[10] All patients underwent extremity-MRI at baseline; of these 113 had 1-year follow-up including radiographs. Patients without 1-year follow-up were less often diagnosed with RA (Suppl Table 2). Of the 113 patients studied, 53 fulfilled the 2010-criteria for RA at baseline. During the first year, three-quarters of the patients were treated with conventional DMARDs.(Table 1) All participants provided written informed consent. The study was approved by the local medical ethics committee.

MRI and radiographs

The MCP2-5, wrist and MTP1-5-joints at the most painful side (or dominant side in case of equally severe symptoms at both sides) were scanned with a 1.5T-extremity-MRI-scanner according to the OMERACT-RAMRIS-protocol. A detailed scan protocol is provided in the supplementary methods. Synovitis and BME were scored according to RAMRIS;[11] teno-

synovitis in MCP-joints and wrists was assessed as described elsewhere.[12] Two readers scored the MR-images independently, blinded to clinical data; the mean scores were studied. Within-reader ICCs for the total MRI-inflammation-score were 0.99 and 0.93; the between-reader ICC 0.87. The total MRI-inflammation was the sum of synovitis, BME and tenosynovitis scores. Subclinical inflammation was defined as inflammation on MRI in clinically non-swollen joints. Radiographs were scored according to the Sharp-van-der-Heijde method (SHS) by one reader in chronological order (ICC baseline SHS 0.86). Radiographic progression was defined as the difference in SHS between year-1 and baseline.

Analyses

First, to replicate previous associations between MRI-inflammation and radiographic progression,[1-7] analyses were done on patient level using a linear regression model with radiographic progression (continuous variable) as outcome. Univariable and multivariable analyses (adjusting for age, gender, ACPA, RF, CRP-level and 66SJC) for total inflammation and each MRI feature separately, were performed. Joints that could not be completely scored on MRI due to insufficient image-quality (1.1% of all individual scores) were imputed with the median value for that feature across all joints or bones within the same patient.

Next, analyses were performed on joint level. For MRI, the sum scores per joint and feature were determined according to RAMRIS. Similarly the sum scores per joint on radiographs were determined according to SHS (Suppl methods). Missing MRI-scores were not imputed at joint level. To compare frequencies of subclinical inflammation the continuous MRI-data was dichotomized; joints with a score ≥1 were considered positive. In sensitivity analyses a cut-off ≥2 was evaluated. Joints that progressed ≥1 SHS-point in 1-year were considered to have radiographic progression. SPSS version 20.0 was used; p-values <0.05 were considered significant.

RESULTS

Total MRI inflammation on patient level

Table 1 presents baseline characteristics of early arthritis and RA-patients. First, we evaluated the total MRI-inflammation scores in relation to radiographic progression and observed that both the total score and the individual MRI-features were associated with radiographic progression in early arthritis and RA, independent of known risk factors ($p<0.05$ except for tenosynovitis in RA, Table 2). We herewith replicated previous observations.[1-7]

Subclinical MRI inflammation on patient level

When separating patients' total MRI-inflammation scores (mean 13.9±12.0) into the scores obtained in swollen joints and the scores obtained in non-swollen joints, the mean score

Table 1 Baseline characteristics 113 early arthritis patients.

	All patients (n=113)	2010-RA patients (n=53)
Age, mean ± SD, years	54.9 ± 15.7	55.4 ± 15.5
Female sex, n (%)	62 (54.9)	32 (60.4)
Symptom duration at inclusion, median (IQR), weeks	15 (7-32.5)	16.5 (8.8-33.3)
Onset of symptoms, n (%)		
(sub) acute	47 (43.1)	20 (38.5)
gradual	61 (56.0)	32 (61.5)
Morning stiffness, median (IQR), minutes	45 (15-82.5)	52.5 (15-112.5)
Swollen joint count, median (IQR)	4 (2-7)	5 (3-10)
Tender joint count, median (IQR)	7 (3-11.5)	8 (4-15.5)
ACPA positivity, n (%)	40 (35.4)	35 (66.0)
RF positivity, n (%)	44 (38.9)	35 (66.0)
CRP level, median (IQR)	6 (3-17.5)	10 (3-19)
ESR level, median (IQR)	19 (6-34)	28 (9-41)
Patient classification at baseline, n (%)		
RA (2010 ACR/EULAR criteria)	53 (46.9)	
Unclassified arthritis (UA)	37 (32.7)	
Psoriatic arthritis	11 (9.7)	
Inflammatory osteoarthritis	4 (3.5)	
Spondyloarthritis	3 (2.7)	
Other diagnoses	5 (4.4)	
Treatment within the first year, n (%)†		
Methotrexate	48 (42.5)	26 (49.1)
Hydroxychloroquine	26 (23.0)	19 (35.8)
Prednisolon orally	17 (15.0)	13 (24.5)
Sulfasalazine	13 (11.5)	11 (20.8)
Trial medication#	8 (7.1)	7 (13.2)
Anti-TNF$^{\infty}$	2 (1.8)	2 (3.4)
Leflunomide	2 (1.8)	1 (1.9)
Azathioprine	1 (0.9)	1 (1.9)
No DMARD	26 (23)	4 (7.5)
MRI BME, median (IQR)	5.5 (2.5-11.8)	6.5 (3.3-15.8)
MRI Synovitis, median (IQR)	4.5 (2.5-7.5)	5.0 (2.3-8.0)
MRI Tenosynovitis, median (IQR)	2.8 (0.5-5.5)	3.5 (0.6-5.5)
MRI Any inflammation, median (IQR)	13.0 (7.6-25.5)	16.8 (8.5-30.1)
SHS, median (IQR)	2.0 (0.0-6.0)	2.0 (0.0-4.0)

At patient level medians (IQR) are shown and missing scores were imputed with the median of non-missing MRI scores of the same feature of the patient. The 66 swollen joint count and 68 tender joint count was assessed.

† The total exceeds100% as some patients used several DMARDs in the first year.

#Trial medication refers to a double-blind randomized trial in which patients received tocilizumab and/or methotrexate.

$^{\infty}$One patient received anti-TNF early in the disease in the IMPROVED trial and the other patient failed methotrexate, hydroxychloroquine and sulfasalazine and then received anti-TNF after 353 days.

Table 2 Associations between baseline MRI inflammation and radiographic SHS progression over the first year.

	Early arthritis patients (n=113)			Subgroup of 2010-RA patients (n=53)		
Univariable:	beta (95% CI)	R^2	P value	beta (95% CI)	R^2	P value
BME	0.06 (0.02-0.10)	0.13	<0.001	0.06 (0.01-0.11)	0.14	0.006
Synovitis	0.14 (0.05-0.22)	0.13	<0.001	0.12 (-0.02-0.23)	0.11	0.02
Tenosynovitis	0.11 (0.03-0.20)	0.08	0.002	0.07 (-0.05-0.21)	0.04	0.19
Total inflammation	0.04 (0.02-0.06)	0.16	<0.001	0.04 (0.01-0.07)	0.15	0.004
Multi-variable:	beta (95% CI)	R^{2a}	P value	beta (95% CI)	R^{2a}	P value
BME	0.05 (0.01-0.09)	0.16	0.003	0.04 (-0.01-0.10)	0.27	0.04
Synovitis	0.13 (0.03-0.21)	0.18	0.001	0.11 (-0.03-0.21)	0.26	0.04
Tenosynovitis	0.10 (0.01-0.17)	0.14	0.011	0.02 (-0.10-0.13)	0.19	0.76
Total inflammation	0.04 (0.01-0.06)	0.18	<0.001	0.03 (-0.01-0.06)	0.26	0.04

* Linear regression analysis was used. Multi-variable: adjusted for age, gender, RF +/-, CCP +/-, CRP level, 66 SJC. R^2 represents the explained variability in radiographic progression, by the model. For the multivariable model the R^2 adjusted (R^{2a}) is presented, which adjusts for the amount of variables included in the model. BME=bone marrow edema. Total inflammation=BME + synovitis + tenosynovitis.

in swollen joints was 7.2±9.4 and in non-swollen joints 6.6±7.8. This suggests that the cumulative amount of MRI-inflammation in non-swollen joints was comparable to that in swollen joints. We subsequently studied non-swollen joints only.

Supplementary Figure 1 showing the number of joints with subclinical inflammation reveals that most patients had 1-3 subclinical inflamed joints and only 9% of patient had no joints with subclinical inflammation. Comparing the number of subclinical inflamed joints between patients with RA and other diagnoses revealed that RA-patients tended to have more joints with subclinical inflammation (2.3 versus 1.9, p=0.11). Evaluating the different inflammation features separately showed that RA-patients mainly had more joints with BME in non-swollen joints (1.6 versus 1.1, p=0.03).

Subclinical inflammation on joint level

Next, we investigated subclinical inflammation at joint level. Of all 1,130 joints studied 932 joints were clinically non-swollen. Of these, 232 (26%) had any subclinical MRI-inflammation: 17% of non-swollen joints had BME, 16% had synovitis and 21% tenosynovitis (Figure 1). Two-percent of the 932 non-swollen joints had radiographic progression during year-1 (compared to 8% of the swollen joints). The non-swollen joints with and without subclinical MRI-inflammation were compared to determine the relative risks (RR) of radiographic progression: 4% of non-swollen joints with any MRI-inflammation had radiographic progression versus 1% of the joints without subclinical inflammation (RR 3.5, 95%CI 1.3-9.6). Similar analyses for the individual MRI-inflammation features revealed that BME in non-swollen joints had the highest RR of radiographic progression (RR 5.3,

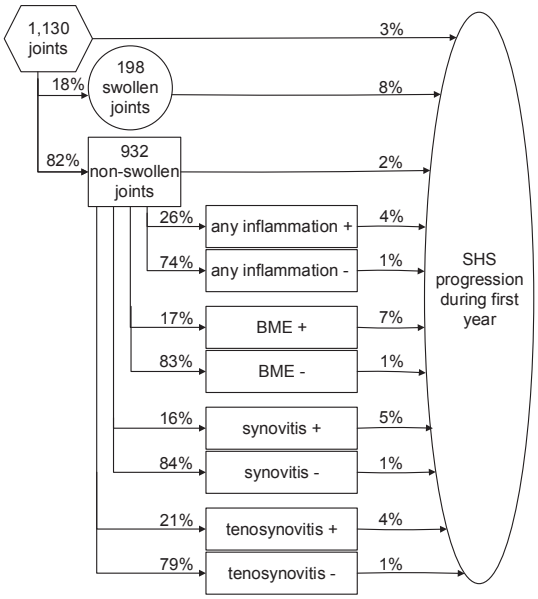


Figure 1 Distribution of MRI inflammation among clinically non-swollen joints and the percentages of joints with radiographic progression during 1-year follow-up.
A joint or bone with a MRI RAMRIS score ≥1 was considered positive. Joints with an increase in SHS of ≥1 were considered to have radiographic progression. The percentages at the right part of the figure represent the absolute risks at radiographic progression in the presence or absence of the MRI feature. Dividing these risks by each other results in the relative risk (RR). These were for any MRI inflammation: RR 3.5 (95%CI 1.3-9.6), BME: RR 5.3 (95%CI 2.0-14.0), synovitis RR 3.4 (95%CI 1.2-9.3) and tenosynovitis RR 3.0 (95%CI 0.7-12.7).
Radiographic progression was detected in 4% of non-swollen joints with MRI inflammation; these joints were present in 11 patients (9.7% of all patients).
At joint level (n=1,130) missing variables were not imputed and not analyzed. Missing variables: BME n=44, synovitis n=18, tenosynovitis n=15.

95%CI 2.0-14.0; 7% versus 1%). For synovitis the RR was 3.4 (95%CI 1.2-9.3; 5% versus 1%). For tenosynovitis the RR was 3.0 (95%CI 0.7-12.7). When repeating these analyses in joints that were non-swollen and also non-tender, the RRs obtained were comparable, although 95%CIs were broader (Suppl. Figure 2). When analyzing wrist joints only, a higher percentage of non-swollen joints with subclinical inflammation was observed (86% versus 26%) and radiographic progression occurred more frequently (11% versus 3%, Suppl. Figure 3). When analyzing radiographic progression in non-swollen hand and foot joints of RA-patients (n=53), the RRs went into the same direction for synovitis and BME, but the 95%CIs were broad.(Suppl. Figure 4) Furthermore, sensitivity analyses with a higher cut-off to define MRI inflammation (≥2 instead of ≥1) revealed similar results (Suppl. Figure 5).

DISCUSSION

This study is the first investigating the radiographic outcome of MRI-detected inflammation in clinically non-inflamed joints at disease presentation. We observed that joints with subclinical inflammation, especially with BME, had an increased risk of radiographic progression during the first year.

Our observation that the total level of MRI-inflammation (in both swollen and non-swollen joints) was an independent predictor for radiographic progression fits with previous findings.[1-7] Notably, the effect sizes of BME and synovitis in our study were similar to those of Boyesen et al. (beta 0.04 and 0.06 for BME and 0.11 and 0.12 for synovitis, respectively).[1] Furthermore, our finding that subclinical inflammation at disease onset is associated with radiographic progression is in line with previous findings on subclinical inflammation in RA-patients in clinical remission.[13-17]

This study has several limitations. First, the sample size, particularly of the RA-group, was moderate. Although the effect sizes obtained in the total group and RA-group were similar, the 95%CIs of the estimates in the RA-group were broad. Second, radiographic progression was infrequent, not only in all early arthritis patients (3% of all joints) but also in RA, a group of patients that is more likely to erode (4%). Third, radiographic progression was defined as ΔSHS on joint level of ≥1,this concerned a small increase but the radiographs were scored in chronological order which reduced the chance on measurement errors. Up-to-date treatment-strategies will have contributed to the low prevalence of radiographic progression. Furthermore, the frequency of progression was determined on joint level, which is relatively uncommon. To obtain a reference, we also analyzed previously SHS-scored unilateral MCP2-5, wrist and MTP1-5 joints of EAC RA-patients included in 1993-1999 and 2000-2006 when different treatment strategies were applied.[10] Here, using the same definition, radiographic progression during year-1 was present in 15% and 9% of joints, suggesting that in the currently studied patients treatment had effectively reduced radiographic progression. Nonetheless, despite radiographic progression being infrequent nowadays, progression was significantly more frequent in joints with subclinical inflammation. This suggests that MRI may be valuable to identify joints with increased risk of progression despite normal physical examination and current treatment-strategies.

This study mainly increases the comprehension of the connection between inflammation and structural damage early in the disease. Whether subclinical MRI-inflammation is relevant to clinical practice remains a question, as rheumatologists treat patients and not joints. Information on subclinical inflamed joints would affect treatment decisions most when patients have few clinically swollen joints. A sub-analysis in patients with ≤2 swollen joints showed a slight tendency towards more progression in the presence of more subclinically inflamed joints (ΔSHS1.4 in case of ≥3 sub-clinically inflamed joints versus ΔSHS

1.0 in case of ≤2 sub-clinically inflamed joints). Larger studies are required to ascertain whether information on MRI-inflammation is relevant for clinical practice.

MRI is a sensitive tool and MRI-inflammation has been reported in symptom-free persons.[18-20] Nevertheless, present data indicate that MRI-detected subclinical inflammation in early arthritis negatively affects radiological outcome.

REFERENCE LIST

1. Boyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011 Mar;70(3):428-33.
2. Haavardsholm EA, Boyesen P, Ostergaard M, et al. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. *Ann Rheum Dis* 2008 Jun;67(6):794-800.
3. Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009 Mar;68(3):384-90.
4. Hetland ML, Stengaard-Pedersen K, Junker P, et al. Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 2010 Oct;69(10):1789-95.
5. Lindegaard HM, Vallo J, Horslev-Petersen K, et al. Low-cost, low-field dedicated extremity magnetic resonance imaging in early rheumatoid arthritis: a 1-year follow-up study. *Ann Rheum Dis* 2006 Sep;65(9):1208-12.
6. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 1999 Mar;58(3):156-63.
7. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003 Jul;48(7):1814-27.
8. Mundwiler ML, Maranian P, Brown DH, et al. The utility of MRI in predicting radiographic erosions in the metatarsophalangeal joints of the rheumatoid foot: a prospective longitudinal cohort study. *Arthritis Res Ther* 2009;11(3):R94.
9. Krabben A, Stomp W, Huizinga TW, et al. Concordance between inflammation at physical examination and on MRI in patients with early arthritis . *Ann Rheum Dis.* In press 2013.
10. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011 Jan;50(1):93-100.
11. Ostergaard M, Edmonds J, McQueen F, et al. An introduction to the EULAR-OMERACT rheumatoid arthritis MRI reference image atlas. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i3-i7.
12. Haavardsholm EA, Ostergaard M, Ejbjerg BJ, et al. Introduction of a novel magnetic resonance imaging tenosynovitis score for rheumatoid arthritis: reliability in a multireader longitudinal study. *Ann Rheum Dis* 2007 Sep;66(9):1216-20.
13. Brown AK, Quinn MA, Karim Z, et al. Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression. *Arthritis Rheum* 2006 Dec;54(12):3761-73.
14. Brown AK, Conaghan PG, Karim Z, et al. An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* 2008 Oct;58(10):2958-67.
15. Gandjbakhch F, Conaghan PG, Ejbjerg B, et al. Synovitis and osteitis are very frequent in rheumatoid arthritis clinical remission: results from an MRI study of 294 patients in clinical remission or low disease activity state. *J Rheumatol* 2011 Sep;38(9):2039-44.
16. Gandjbakhch F, Foltz V, Mallet A, et al. Bone marrow oedema predicts structural progression in a 1-year follow-up of 85 patients with RA in remission or with low disease activity with low-field MRI. *Ann Rheum Dis* 2011 Dec;70(12):2159-62.

17. Gandjbakhch F, Haavardsholm EA, Conaghan PG, et al. Determining a Magnetic Resonance Imaging Inflammatory Activity Acceptable State Without Subsequent Radiographic Progression in Rheumatoid Arthritis: Results from a Followup MRI Study of 254 Patients in Clinical Remission or Low Disease Activity. *J Rheumatol* 2013 Dec 15.
18. Ejbjerg B, Narvestad E, Rostrup E, et al. Magnetic resonance imaging of wrist and finger joints in healthy subjects occasionally shows changes resembling erosions and synovitis as seen in rheumatoid arthritis. *Arthritis Rheum* 2004 Apr;50(4):1097-106.
19. Olech E, Crues JV III, Yocum DE, et al. Bone marrow edema is the most specific finding for rheumatoid arthritis (RA) on noncontrast magnetic resonance imaging of the hands and wrists: a comparison of patients with RA and healthy controls. *J Rheumatol* 2010 Feb;37(2):265-74.
20. Xie X, Webber CE, Adachi JD, et al. Quantitative, small bore, 1 Tesla, magnetic resonance imaging of the hands of patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2008 Sep;26(5):860-5.

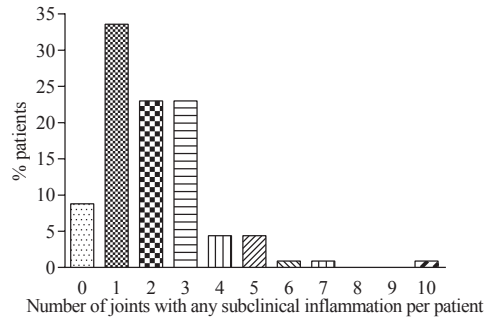
Supplementary Table 1 Summary of previous studies in RA on baseline MRI and radiographic progression

Reference	N	MRI	Outcome	Main result
Haavardsholm et al.(2)	84	Unilateral wrist	Radiographic progression in hands after 1-year	BME and sex independent predictors
Boyesen et al.(1)	55	Unilateral wrist	Radiographic progression in hands after 3-years	ESR , MRI synovitis, MRI BME independent predictors
Hetland et al.(3)	130	Unilateral wrist and MCPs	Radiographic progression in hands and feet after 2-years	BME independent predictor
Hetland et al.(4)	139	Unilateral wrist and MCPs	Radiographic progression in hands and feet after 5-years	ACPA, BME and baseline radiographic damage independent predictors
McQueen et al.(6)	42	Unilateral wrist	Radiographic progression in hands and feet after 1-year	Total MRI score>13, MRI erosions and ESR
McQueen et al.(7)	31	Unilateral wrist	Radiographic progression in hands and feet after 6-years	BME, pain score, CRP, ESR and baseline radiographic damage independent predictors
Lindegaard et al.(5)	24	Unilateral wrist and MCPs	Radiographic erosion progression in hands after 1-year	MRI erosions and BME predictors
Mundwiler et al.(8)	50	MTP3-5	Radiographic progression in MTP3-5 after 1-year	Almost no progression without BME

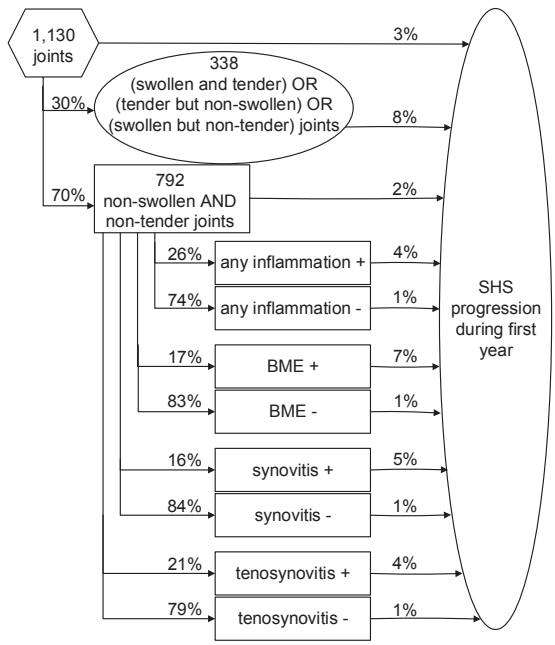
Supplementary Table 2 Baseline characteristics of 113 early arthritis patients with 1 year radiographs and 66 early arthritis patients without 1 year radiographs

	Patients with 1 year radiographs (n=113)	Patients without 1 year radiographs (n=66)	P value
Age, mean ± SD, years	54.9 ± 15.7	51.9 ± 14.3	0.11
Female sex, n (%)	62 (54.9)	37 (56.1)	0.88
Symptom duration at inclusion, median (IQR), weeks	15 (7-32.5)	13 (5-24)	0.10
Morning stiffness, median (IQR), minutes	45 (15-82.5)	15 (15-60)	0.01
Swollen joint count, median (IQR)	4 (2-7)	2 (1-4)	<0.001
Tender joint count, median (IQR)	7 (3-11.5)	5 (1-11)	0.11
ACPA positivity, n (%)	40 (35.4)	5 (7.7)	<0.001
RF positivity, n (%)	44 (38.9)	10 (15.4)	0.001
CRP level, median (IQR)	6 (3-17.5)	3 (3-7)	0.001
ESR level, median (IQR)	19 (6-34)	10 (6-22)	0.04
Patient classification at baseline, n (%)			
RA (2010 ACR/EULAR criteria)	53 (46.9)	13 (19.7)	<0.001
Unclassified arthritis (UA)	37 (32.7)	28 (42.4)	
Psoriatic arthritis	11 (9.7)	4 (6.1)	
Inflammatory osteoarthritis	4 (3.5)	8 (12.1)	
Spondyloarthritis	3 (2.7)	0 (0)	
Other diagnoses	5 (4.4)	13 (19.7)	
MRI BME, median (IQR)	5.5 (2.5-11.8)	3.5 (1.5-6.0)	0.001
MRI Synovitis, median (IQR)	4.5 (2.5-7.5)	3.0 (1.5-5.6)	0.01
MRI Tenosynovitis, median (IQR)	2.8 (0.5-5.5)	1.5 (0.4-30.0)	0.01
MRI Any inflammation, median (IQR)	13.0 (7.6-25.5)	8.8 (4.5-14.0)	0.001
SHS, median (IQR)	2.0 (0.0-6.0)	0.0 (0.0-3.0)	0.004

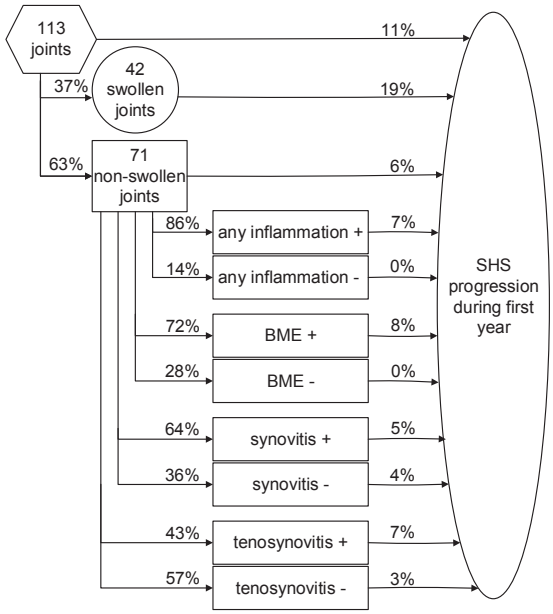
Of the 179 EAC patients with MRI; 113 patients had a radiograph at one year of follow-up and 66 patients not.



Supplementary Figure 1 Distribution of the number of joints with any subclinical inflammation per patient.
A joint with any subclinical inflammation, is a non-swollen joint with a MRI bone marrow edema (BME) score ≥1 and/or synovitis score ≥1 and/or tenosynovitis score ≥1.

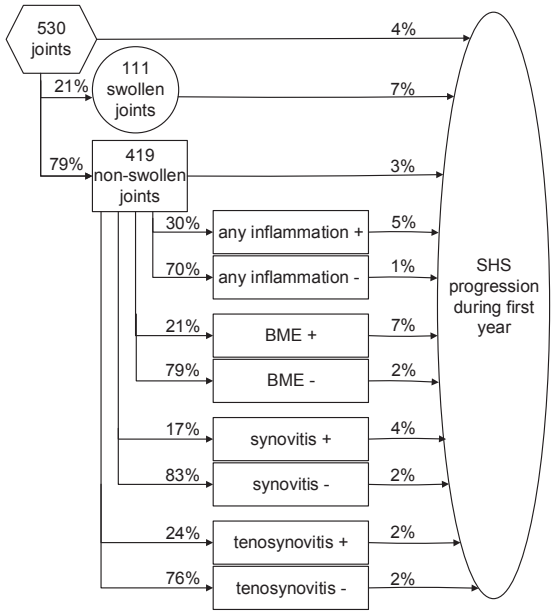


Supplementary Figure 2 Distribution of MRI inflammation among clinically non-swollen and non-tender joints in all early arthritis patients and the percentages of joints with radiographic progression during 1-year follow-up.
A joint or bone with a MRI RAMRIS score ≥1 was considered positive. Joints with an increase in SHS of ≥1 were considered to have radiographic progression. The percentages at the right part of the figure represent the absolute risks at radiographic progression in the presence or absence of the MRI feature. Dividing these risks by each other results in the relative risk (RR). These were for any MRI inflammation: RR 2.6 (95%CI 0.9-7.7), BME: RR 4.2 (95%CI 1.5-11.8), synovitis RR 1.8 (95%CI 0.5-6.5) and tenosynovitis RR 2.2 (95%CI 0.4-11.7).



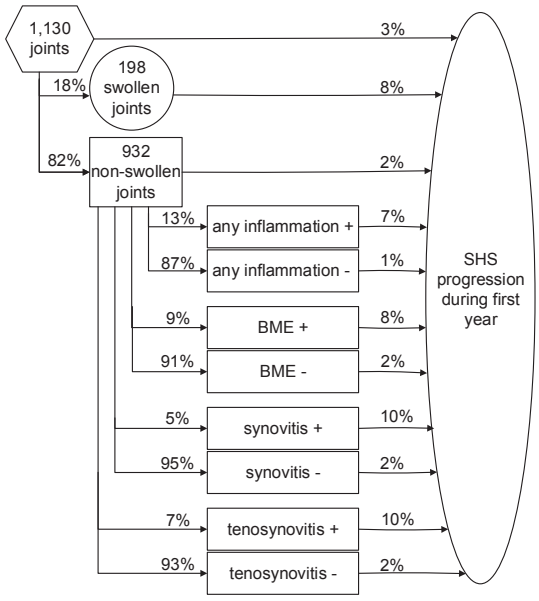
Supplementary Figure 3 Distribution of MRI inflammation among clinically non-swollen wrist joints in all early arthritis patients and the percentages of joints with radiographic progression during 1-year follow-up

A joint or bone with a MRI RAMRIS score ≥1 was considered positive. Joints with an increase in SHS of ≥1 were considered to have radiographic progression. The percentages at the right part of the figure represent the absolute risks at radiographic progression in the presence or absence of the MRI feature. Dividing these risks by each other results in the relative risk (RR). These were 'infinite" for any MRI inflammation and BME because of 0% progression in the joints/bones without MRI inflammation or without BME. For synovitis and tenosynovitis the RRs were 1.1 (95%CI 0.1-11.9) and 2.6 (95%CI 0.2-27.3), respectively.



Supplementary Figure 4 Distribution of MRI inflammation among clinically non-swollen joints and the percentages of joints with radiographic progression during 1-year follow-up, in a subgroup of RA patients.

A joint or bone with a MRI RAMRIS score ≥1 was considered positive. Joints with an increase in SHS of ≥1 were considered to have radiographic progression. The percentages at the right part of the figure represent the absolute risks at radiographic progression in the presence or absence of the MRI feature. Dividing these risks by each other results in the relative risk (RR). These were for any MRI inflammation: RR 3.6 (95%CI 0.9-14.9), BME: RR 4.5 (95%CI 1.2-16.7), synovitis RR 2.1 (95%CI 0.4-8.7) and tenosynovitis RR 1.1 (95%CI 0.04-11.3).



Supplementary Figure 5 Sensitivity analyses: Distribution of MRI inflammation, defined as a score of ≥2, among clinically non-swollen joints and the percentages of joints with radiographic progression during 1-year follow-up.

A joint or bone with a MRI RAMRIS score ≥2 was considered positive. Joints with an increase in SHS of ≥1 were considered to have radiographic progression. The percentages at the right part of the figure represent the absolute risks at radiographic progression in the presence or absence of the MRI feature. Dividing these risks by each other results in the relative risk (RR). These were for any MRI inflammation: RR 5.2 (95%CI 1.9-13.9), BME: RR 4.8 (95%CI 1.7-13.1), synovitis RR 6.1 (95%CI 1.7-18.6) and tenosynovitis RR 6.7 (95%CI 1.4-28.7). As a result of using the higher cut-off score of 2, the number of joints positive for MRI inflammation declined and the data should be interpreted with caution. (BME positive joints n=78, synovitis positive joints n=41, tenosynovitis positive joints n=30, total inflammation positive joints n=119)

SUPPLEMENTARY METHODS

MRI scan protocol

MR imaging of the hand (wrist and metacarpophalangeal joints) and forefoot (metatarsophalangeal joints) was performed within two weeks after inclusion, at the most painful side, or in case of completely symmetric symptoms at the dominant side. The presence of clinical arthritis at physical examination of the joints that were scanned was not a prerequisite. Two patients were excluded because of contraindications for MR imaging. Patients with impaired renal function or known hypersensitivity or allergic reactions to contrast media were imaged without contrast administration (n=2).

MR imaging was performed on a MSK-extreme 1.5T extremity MR imaging system (GE, Wisconsin, USA) using a 145mm coil for the foot and a 100mm coil for the hand. The patient was positioned in a chair beside the scanner, with the hand or foot fixed in the coil with cushions.

The forefoot was scanned using a T1-weighted fast spin-echo (FSE) sequence in the axial plane with repetition time (TR) of 650 ms, echo time (TE) 17ms, acquisition matrix, 388×288, echo train length (ETL) 2; and a T2-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 3000/61.8; acquisition matrix 300x224, ETL7). Due to time constraints, imaging of the foot was limited to pre-contrast sequences only.

In the hand, the following sequences were acquired before contrast injection: T1-weighted FSE sequence in the coronal plane (TR/TE 650/17ms; acquisition matrix 388×88; ETL2); T2-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 3000/61.8ms; acquisition matrix, 300x224, ETL7). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 650/17ms, acquisition matrix 364×224, ETL2), T1-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 570/7 ms; acquisition matrix 320x192; ETL2).

Field-of-view was 100mm for the hand and 140mm for the foot. Coronal sequences had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. All axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm, with 20 slices for the hand and 16 for the foot. Total imaging time was approximately 75 minutes.

The inter-reader reliability was assessed by computing the interclass correlation for total scores of each MR imaging parameter. In addition, a subset of 25 randomly selected MR image sets (14%) was scored twice by each reader to determine intra-reader ICC's. Intra-reader ICCs for synovitis were 0.93 for reader 1 and 0.64 for reader 2 and interreader ICC 0.65, for tenosynovitis 0.91, 0.93 and 0.90, for bone marrow edema 0.96, 0.72 and 0.86, respectively.

Summing of MRI scores and SHS at joint level

To allow comparisons of MRI inflammation and radiographic joint damage at joint level several inflammation features were summed. According to RAMRIS, proximal and distal bones of MCP and MTP joints were scored separately for BME, these were summed per joint. For the wrist, 15 bones were scored for BME; these scores were summed for the wrist score. For MRI synovitis, the scores in the MCP and MTP joints were straightforward, in the wrist three joints (distal radio-ulnar, radio-carpal and intercarpal joint) were scored and summed to obtain the synovitis score in the wrist. For tenosynovitis in the MCP and wrist joints, the scores of the flexor and extensor sites were summed. Also for the SHS scores at joint level, the scores of erosions and joint space narrowing of the wrist that were assessed according to the Sharp van der Heijde scoring method were summed.

12

Are rheumatoid arthritis patients discernible from other early arthritis patients using 1.5 extremity MRI?: a large cross-sectional study.

W. Stomp, A. Krabben, D. van der Heijde, T. W. J. Huizinga, J. L. Bloem, A. H. M. van der Helm-van Mil, M. Reijnierse

Journal of Rheumatology, 2014 August;41(8):1630-1637.

ABSTRACT

Objective

MRI is increasingly used in Rheumatoid arthritis (RA) research. A EULAR task-force recently suggested that MRI can improve the certainty of the diagnosis RA. Since this recommendation may reflect a tendency to use MRI in daily practice, thorough studies on the value of MRI are required. Thus far no large studies have evaluated the accuracy of MRI to differentiate early RA from other early arthritis patients. We therefore performed a large cross-sectional study to determine if patients that are clinically classified with RA differ in MRI features compared to patients with other diagnoses.

Methods

179 patients presenting with early arthritis (median symptom duration 15.4 weeks) underwent 1.5T-extremity MR-imaging of unilateral wrist, metacarpophalangeal and metatarsophalangeal joints according to our arthritis protocol, the foot without contrast. Images were scored according to OMERACT RAMRIS by two independent readers. Tenosynovitis was also assessed. The main outcome was fulfilling the 1987 ACR-criteria for RA. Test characteristics and areas under the receiver-operator-characteristic-curves (AUCs) were evaluated. In sub-analyses the 2010-ACR/EULAR-criteria were used as outcome and analyses were stratified for ACPA.

Results

The ACR87-criteria were fulfilled in 43 patients (24.0%). RA-patients had higher scores for synovitis, tenosynovitis and bone marrow edema (BME) than non-RA patients ($p<0.05$). ACPA-positive patients had more BME (median scores 6.5 vs. 4.25, $p=0.016$) than ACPA-negative patients. For all MRI features the predictive value for the presence of RA was low (PPV <50%). For all MRI features the AUCs were <0.70. 2010+1987- patients had less synovitis than 2010+1987+ patients ($p=0.029$)

Conclusion

Although RA-patients had higher scores of MRI-inflammation and ACPA-positive patients had more BME, the severity of MRI-inflammation assessed according to RAMRIS does not accurately differentiate RA-patients from other early arthritis patients.

INTRODUCTION

Early identification of rheumatoid arthritis (RA) is important because early initiation of aggressive treatment results in a better outcome.[1] However, this requires that RA-patients are identified amongst other early arthritis patients. Magnetic Resonance Imaging (MRI) in RA is presently mainly used for research purposes. The value of MRI is supported by its sensitivity to depict changes that are not detectable by physical examination and the association of bone marrow edema (BME) with radiographic progression over time.[2] A recent EULAR taskforce recommended that "in case of diagnostic doubt, MR imaging can improve the certainty of a diagnosis of RA".[3] Since this recommendation may reflect a tendency to use MRI in daily practice, thorough studies on the value of MRI in a general setting of early arthritis patients are required. Thus far no large studies evaluated the accuracy of MRI to differentiate RA-patients from early arthritis patients with other diagnoses. The majority of studies performed on the diagnostic accuracy primarily evaluated patients with undifferentiated arthritis or RA but not the entire spectrum of early arthritis patients.[4,5] Furthermore, they included a low number of early arthritis patients (less than 50) and reported variable test characteristics (the sensitivity and specificity of certain MR imaging findings ranged between 20–100% and 0–100%). Therefore at present the accuracy to differentiate RA patients from other patients with early arthritis is unclear. We performed a large cross-sectional study to determine this. The outcome was the diagnosis according to classification criteria at two weeks. On purpose we did not explore the additional value of MRI when added to clinical diagnoses, but we started with addressing an even more basic question, being whether patients that are clinically classified with RA differ in MRI features compared to patients with other diagnoses. Because these patients are clinically clearly distinctive, amongst other things in the joints that are typically involved and the extent of inflammation, we anticipated finding differences at 1.5T extremity MRI of the joints most frequently involved in RA. Also, these findings will serve as a basis for further future analyses in the current cohort of patients.

MATERIALS AND METHODS

Patients

Patients were included in the Leiden Early Arthritis Clinic (EAC). Inclusion required the presence of clinically confirmed arthritis of ≥1 joint and symptoms for ≤2-years. Parameters collected at inclusion were medical history, questionnaires, joint counts, laboratory tests, and radiographs of hands and feet. For a detailed description see reference.[6] Anti-citrullinated-peptide-antibodies (ACPA) were measured (anti-CCP2; Eurodiagnostica, Arnhem, The Netherlands). After two weeks, when the laboratory results were known, patients

were diagnosed with RA or other diagnoses according to existing classification criteria, blinded to MR findings. RA was classified according to the 1987-criteria; in sub-analyses RA according to the 2010-criteria was also studied as outcome. These cross-sectional data were studied.

From August 2010 until April 2012, 350 patients were included in the EAC. MR imaging was performed in 179 patients based on voluntary participation. The patients with and without MR did not significantly differ in age, sex, symptom duration or ACPA status (data not shown). The study was approved by the local ethical committee. All patients signed informed consent.

MR imaging

MR imaging of the hand (wrist and metacarpophalangeal joints) and forefoot (metatarsophalangeal joints) was performed within two weeks after inclusion, at the most painful side, or in case of completely symmetric symptoms at the dominant side. The presence of clinical arthritis at physical examination of the joints that were scanned was not a prerequisite. Two patients were excluded because of contraindications for MR imaging. Patients with impaired renal function or known hypersensitivity or allergic reactions to contrast media were imaged without contrast administration (n=2).

MR imaging was performed on a MSK-extreme 1.5T extremity MR imaging system (GE, Wisconsin, USA) using a 145 mm coil for the foot and a 100 mm coil for the hand. The patient was positioned in a chair beside the scanner, with the hand or foot fixed in the coil with cushions.

The forefoot was scanned using a T1-weighted fast spin-echo (FSE) sequence in the axial plane with repetition time (TR) of 650 ms, echo time (TE) 17 ms, acquisition matrix, 388×288, echo train length (ETL) 2; and a T2-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 3000/61.8; acquisition matrix 300x224, ETL 7). Due to time constraints, imaging of the foot was limited to pre-contrast sequences only.

In the hand, the following sequences were acquired before contrast injection: T1-weighted FSE sequence in the coronal plane (TR/TE 650/17 ms; acquisition matrix 388×88; ETL 2); T2-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 3000/61.8 ms; acquisition matrix, 300x224, ETL 7). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 650/17 ms, acquisition matrix 364×224, ETL2), T1-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 570/7 ms; acquisition matrix 320x192; ETL 2).

Field-of-view was 100 mm for the hand and 140 mm for the foot. Coronal sequences had 18 slices with a slice thickness of 2 mm and a slice gap of 0.2 mm. All axial sequences

had a slice thickness of 3 mm and a slice gap of 0.3 mm, with 20 slices for the hand and 16 for the foot. Total imaging time was approximately 75 minutes.

MR imaging scoring

MR images were scored by two readers (WS and AK), blinded to clinical data. Each reader separately analyzed each set of images and the mean total scores for each feature of both readers were used for further analyses. Synovitis, bone marrow edema and erosions were scored semi-quantitatively according to OMERACT RAMRIS definitions and score. Tenosynovitis in the MCP and wrist joints was evaluated using the method proposed by Haavardsholm et al., with tenosynovitis assessed for the flexor and extensor tendons of each MCP joint at the same 0-3 scale as for the wrist.[7] Tenosynovitis was not assessed in the foot because of the lack of axial images.

Total RAMRIS score was defined as the total of all scores including tenosynovitis. Some joints could not be completely scored due to insufficient image quality (1.1% of all individual scores), in most cases due to incomplete fat suppression or movement artifacts. In these cases values were imputed with the median value for that feature across all joints or bones within the same patient. The inter-reader reliability was assessed by computing the intra-class correlation for total scores of each MR imaging parameter. In addition, a subset of 25 randomly selected MR image sets (14.0%) was scored twice by each reader to determine intra-reader ICC's. Intra-reader ICCs for total RAMRIS-score were 0.98 for reader 1 and 0.83 for reader 2 and inter-reader ICC for total RAMRIS-score was 0.89. For synovitis intra-reader ICCs were 0.93 and 0.64 and inter-reader ICC 0.65, for tenosynovitis 0.91, 0.93 and 0.90, for bone marrow edema 0.96, 0.72 and 0.86 and for erosions 0.89, 0.65 and 0.76 respectively.

Statistical analysis

The Wilcoxon Rank Sum and Kruskal-Wallis tests were used where appropriate. To evaluate the discriminative ability of MRI the area under the receiver-operating-characteristic-curves (AUC), test characteristics and positive and negative likelihood ratio (LR+, LR-) were assessed. Optimal cut-off points for dichotomization were determined per MRI feature using Youden's method.[8] Analyses were performed using R, version 2.15.0 (R Development Core Team). P-values <0.05 were considered significant.

RESULTS

Patient characteristics

Of the 179 patients, 99 were female (55.3%). The median age was 57 years (IQR 20), the median symptom duration 15.4 weeks (IQR 21) and 45 (25.1%) of the patients were

ACPA-positive. Patients were classified according to the following diagnoses: 1987-RA 43 (24.0%), UA 88 (49.2%), inflammatory osteoarthritis 12 (6.7%), psoriatic arthritis 15 (8.4%) and other rheumatic diagnoses 21 (11.7%). The patient characteristics per diagnosis are presented in Table 1.

Table 1. Patient characteristics per diagnosis.

Characteristic	RA (n=43)	UA (n=88)	OA (n=12)	PsA (n=15)	Other (n=21)
Age, yrs, median (IQR)	59 (24)	55 (20)	62.5 (9)	47.5 (14)	52.5 (33)
Sex (women/men)	23/20	52/36	7/5	6/9	11/10
Symptom duration, weeks, median (IQR)	17.3 (28.2)	10.7 (20)	33.8 (78.5)	30.9 (32.9)	10.6 (17.3)
Rheumatoid factor positivity, n(%)	27 (62.8)	21 (23.9)	0 (0.0)	4 (26.7)	2 (9.5)
ACPA positivity, n(%)	21 (48.8)	20 (22.7)	0 (0.0)	3 (20.0)	1 (4.8)
CRP, mg/l, median (IQR)	8 (19)	4 (5)	3 (1)	4 (10)	12 (23)
66 Swollen joint count, median (IQR)	7 (8)	2 (3)	3 (3)	5 (3)	2 (4)

RA: rheumatoid arthritis according to the 1987 ACR-criteria, UA: undifferentiated arthritis, OA: inflammatory osteoarthritis, PsA: psoriatic arthritis, other: other rheumatic diagnoses including reactive arthritis (n=6), spondylarthropathy (n=3) , gout (n=2), pseudogout (1), palindromic arthritis (n=1), paramalignant arthritis (n=1), lyme disease (n=1), systemic lupus erythematosus (n=1) RS3PE (n=1), sarcoidosis (1) and unspecified other (n=3).

MRI scores per group of diagnoses

The median scores for synovitis, bone marrow edema, erosions and tenosynovitis per joint group are presented in Table 2. Figure 1 depicts the scores for patients with different diagnoses. Scores for synovitis, tenosynovitis and bone marrow edema seemed higher in RA-patients than in early arthritis patients with other diagnoses (Figure 1). Subsequently we tested whether patients with RA had different MR imaging results than patients with other diagnoses. These differences were statistically significant when comparing RA with all patients with other diagnoses. The median scores for RA and other diagnoses were respectively 5.5 and 4.0 for synovitis (p=0.003) 3.0 and 1.5 for tenosynovitis (p=0.005), and 6.5 and 4.5 for bone marrow edema (p=0.038). The erosion scores were not statistically significantly different (4.5 and 3.5 for RA and other diagnoses respectively, p=0.15).

Accuracy of MR imaging in differentiating RA from other diagnoses

Next the accuracy to differentiate RA from patients with other diagnoses was evaluated by determining the test characteristics and the AUC (Table 2). In the presence of a certain MRI feature, the chance that this patient had RA was low (low positive predictive value). The AUCs of all features were lower than 0.70.

Table 2. Median values for the total RAMRIS and the individual features and the diagnostic accuracy to differentiate RA from other diagnoses per MRI feature

	Total	Wrist	MCP	MTP	Sensitivity	Specificity	PPV	NPV	LR+	LR-	AUC
Synovitis	4.0 (5.0)	1.5 (3.0)	1.0 (2.5)	0.5 (1.0)	93%	27%	0.29	0.92	1.27	0.26	0.63
Tenosynovitis	2.0 (4.0)	1.0 (3.0)	0.5 (2.0)	n.a.	65%	63%	0.36	0.85	1.76	0.56	0.62
BME	4.5 (6.5)	2.0 (5.0)	0.5 (1.5)	0.5 (1.5)	37%	84%	0.42	0.81	2.31	0.75	0.61
Erosions	3.5 (4.0)	2.5 (3.0)	0.5 (1.0)	0.5 (1.0)	40%	78%	0.36	0.64	1.82	0.77	0.60

Scores per feature by joint area, median (IQR) values. BME = bone marrow edema. PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; AUC = Area under the curve. The cut-off to dichotomize the scores were 1.75 for synovitis, 2.75 for tenosynovitis, 10.50 for BME and 5.75 for erosions

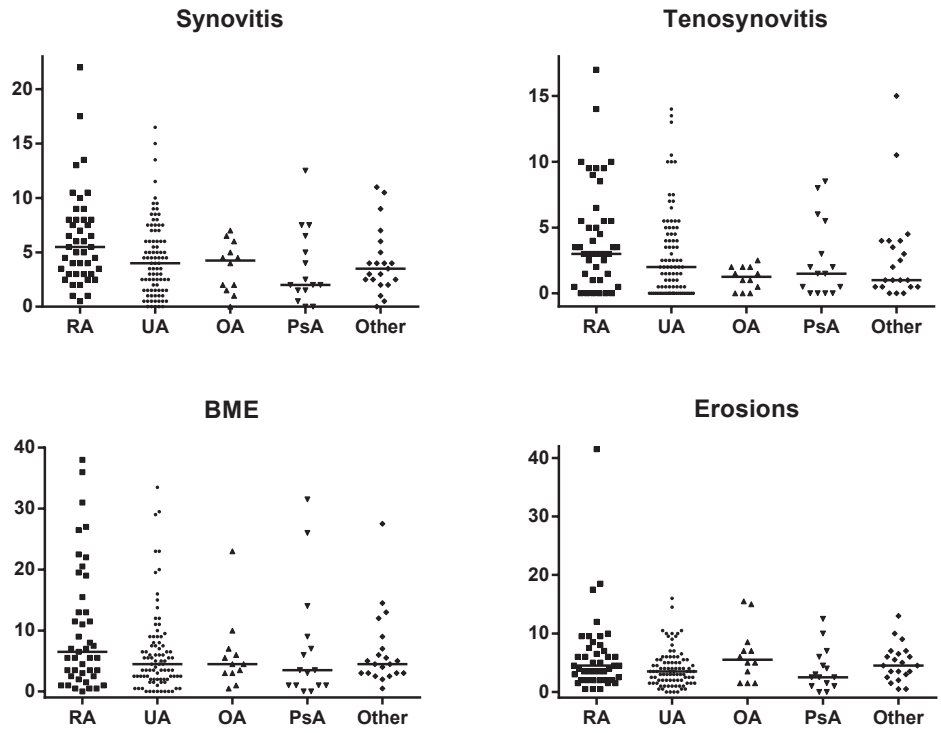


Figure 1: RAMRIS-scores for the different MRI features per group of diagnoses
Horizontal lines represent median values. RA: rheumatoid arthritis according to the 1987 ACR-criteria, UA: undifferentiated arthritis, OA: inflammatory osteoarthritis, PsA: psoriatic arthritis, other: other rheumatic diagnoses including reactive arthritis (n=6), spondylarthropathy (n=3), gout (n=2), pseudogout (1), palindromic arthritis (n=1), paramalignant arthritis (n=1), lyme disease (n=1), systemic lupus erythematosus (n=1) RS3PE (n=1), sarcoidosis (1) and unspecified other (n=3). Total RAMRIS: sum of synovitis, tenosynovitis, BME and erosion scores.

RA according to the 1987ACR or 2010 ACR/EULAR criteria

We subsequently questioned whether the results would be different when the 2010 criteria would be used to classify RA. Analyses were repeated with 2010-RA as outcome, yielding similar results (Supplementary Figure 1). As many patients classified positive on both criteria sets, we also compared RA-patients that were 1987+/2010+ (n=34), 1987+2010- (n=9) and 1987-/2010+ (n=32) (patients with clear diagnoses other than RA and UA were not included). This showed that 1987-/2010+patients had lower synovitis scores (median 3.25 versus 6.0, p=0.029) than 1987+/2010+ patients (Supplementary Figure 2). No differences were found between 1987+/2010+ and 1987+/2010-RA.

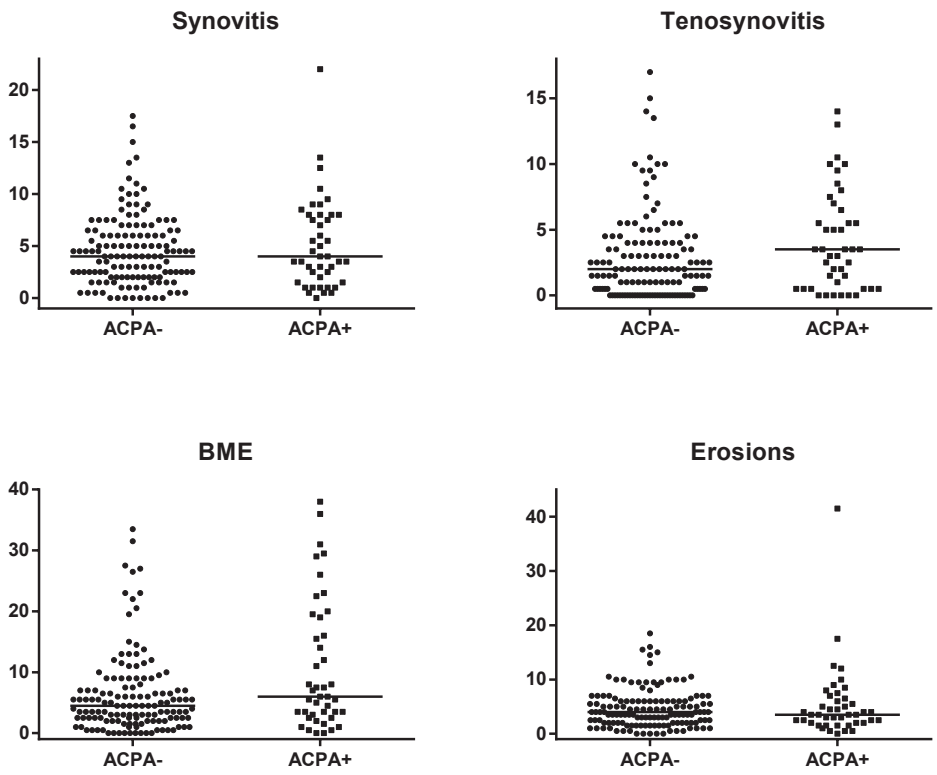


Figure 2: RAMRIS-scores for the different MRI features per group of diagnoses for RA and UA patients with and without ACPA (n=39 and 92 respectively).
Horizontal lines represent median values. For tenosynovitis in the ACPA-negative group many scores are clustered at 0. Synovitis p=0.57, tenosynovitis p=0.40, BME p=0.017, erosions p=0.93.

ACPA-positive versus ACPA-negative arthritis

Next we evaluated whether RA or UA-patients (according to the 1987-criteria) with (n=39) or without ACPA (n=92) had differences in scores. ACPA-positive patients showed higher scores for BME (median 6.5) than ACPA-negative patients (median 4.25, p=0.016). However, no differences in the extend of synovitis, tenosynovitis and erosions scores were observed (Figure 2).

Value of hand and foot joints

The RAMRIS is developed for wrists and MCP joints. We also performed MR imaging of the forefoot. When we evaluated the scores of hands and feet separately, it was observed that the scores in the feet were lower (Table 2), but that the distributions of the scores of hands and feet among the different diagnoses were comparable (Supplementary Figure 3). Also

when the test characteristics were determined with and without the feet, similar results were obtained (data not shown).

DISCUSSION

Many questions remain to be answered before it can be decided whether 1.5T extremity MRI is valuable for use in clinical practice in the field of RA. One of these is a basic question, namely whether the abnormalities seen on MRI are different in patients with RA compared to early arthritis patients with other diagnoses. The present cross-sectional study set out to explore this, making use of an unselected set of early arthritis patients. It was observed that among all patients presenting with early arthritis, patients with RA had significantly higher synovitis, bone marrow edema and tenosynovitis scores than patients without RA, but also that high synovitis, bone marrow edema and tenosynovitis scores were not confined to patients diagnosed with RA. Consequently, the ability of MR imaging to differentiate RA from non-RA patients was low.

In this study we did not focus on the subset of patients with UA. The number of UA-patients was relatively low and follow-up data were not yet available. The definite diagnosis of these patients can be established after 1 or 2 year time. The present study addressed a basic issue by evaluating which differences in MRI features occur between patients with different diagnoses. Although several statistically significant differences were found, RA patients did not have striking differences in the severity of MRI inflammatory scores. Furthermore, in the presence of a certain MRI feature the chance that this patient had RA was low (low positive predictive value). As the undifferentiated arthritis patients group included patients that will go on to develop rheumatoid arthritis and other diagnoses, results may differ when final diagnoses are used to define groups. Particularly, prospective studies are required to determine whether MRI is valuable for classification of patients that are clinically undefined. Follow-up of the studied cohort of patients is currently underway and will be presented in future studies.

Our study has several limitations. The joints scanned are the joint regions that are most commonly involved in RA, also the RAMRIS method was developed for RA. Patients with other diagnoses may have abnormalities in structures that were not scanned or scored, being for instance inflammation in other joints or capsulitis. When a protocol would be developed for use in practice in RA patients, the joints as assessed here will likely be included. Adding other small joints, for instance interphalangeal joints or other structures may possibly enhance the discriminative ability. This is subject for further studies.

One strength of our study was that we scanned MTP joints in addition to the more often assessed wrist and MCP joints. This seems relevant because foot involvement is common in early RA and abnormalities may be found even when the hand MR imaging results are nor-

mal.[9,10] Unfortunately time constraints prohibited the addition of axial and post-contrast imaging of the foot. However the contribution of the foot to total scores was generally low. This was not only true for synovitis, for which the lack of gadolinium contrast might have decreased sensitivity, but also for bone marrow edema and erosions. The MRI features were similarly distributed in hand and foot. Thus findings from this study do not support routine inclusion of MRI of the foot and hand/wrist MRI is probably adequate, however studies with a more complete assessment of the MTP joints including post-contrast imaging are necessary for a more definite recommendation.

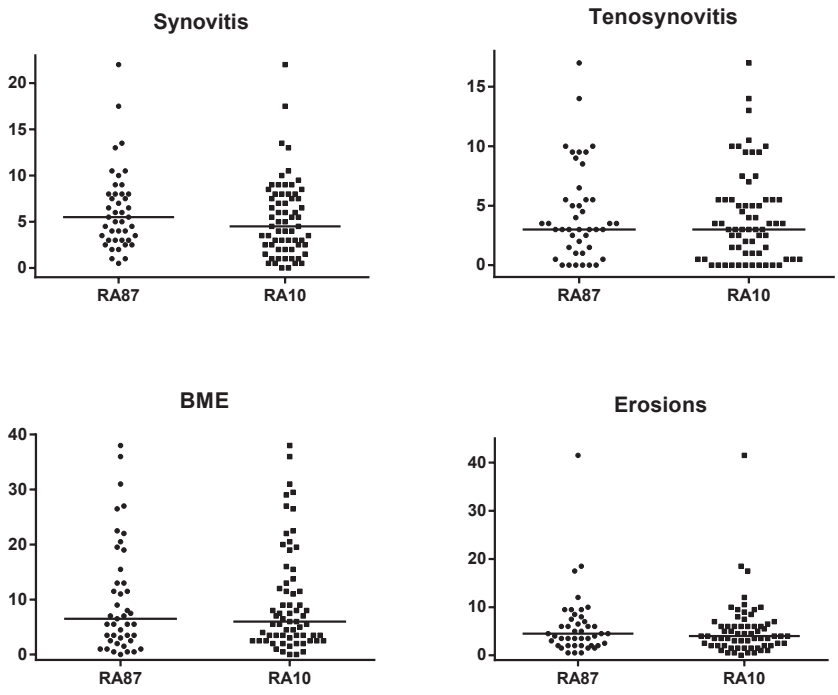
Although many clinical studies have been performed comparing the 1987 and 2010 criteria for RA, to the best of our knowledge no MRI studies on this subject have been published. We observed no difference in MRI scores between RA when classifying RA according to the 1987 ACR-criteria or the 2010ACR/EULAR-criteria. However a majority of patients overlapped between these two groups. When assessing the patients that were positive for both or for one of these sets of criteria separately, we did observe that RA-patients fulfilling 2010 criteria but not the 1987-criteria had less synovitis. These baseline MRI data suggest that patients that only fulfill the 2010-criteria have a milder disease; an observation which is in line with the results of studies comparing the long-term outcome of RA when using the different classification criteria for RA.[11]

Because it has been suggested that ACPA+ and ACPA- disease are separate entities of RA,[12] we performed stratified analyses. ACPA-positive patients had significantly more BME than ACPA-negative patients. As BME is a predictor for progression of joint destruction,[3] this observation is in line with ACPA-positive RA being a more severe disease. Only one earlier study has explored the relation between ACPA and BME, also reporting a significantly higher proportion of patients with BME in the ACPA+ group.[13] Furthermore, subclinical inflammation including bone marrow edema has been observed in ACPA positive arthralgia patients (although no ACPA- control group was present in that study).[14] This observation also relates to the recent observation that ACPA may be able to directly activate osteoclasts.[15] Altogether these data support the use of MRI to further increase the understanding of the relation between these two risk markers for severe RA, as MRI is the only imaging modality able to show BME.

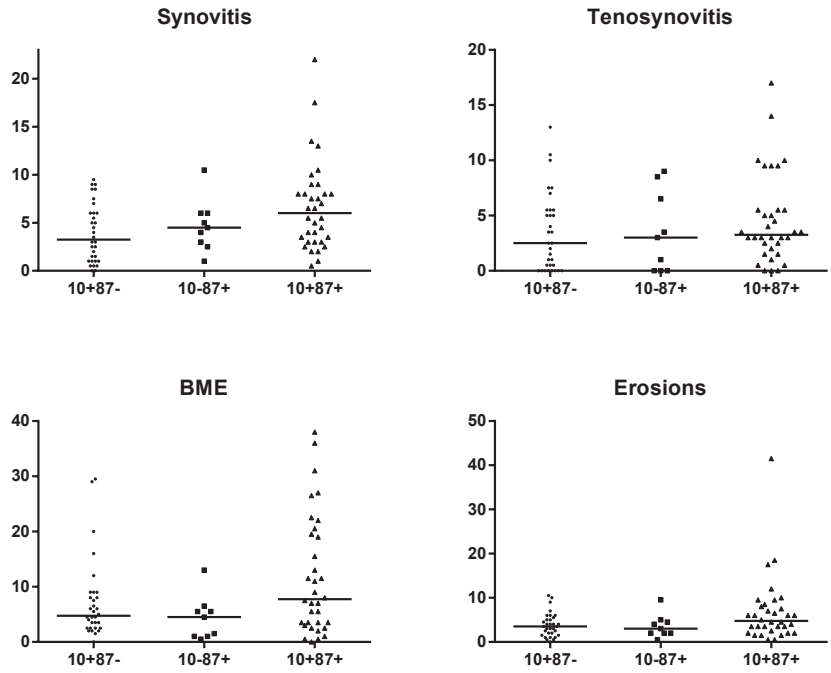
In conclusion, MRI inflammatory scores were higher in RA than in other diagnoses and ACPA-positive patients had more BME than ACPA-negative patients. Nonetheless, the severity of MRI-inflammation assessed according to RAMRIS does not accurately differentiate patients fitting ACR criteria for RA at one time point from other early arthritis patients.

REFERENCE LIST

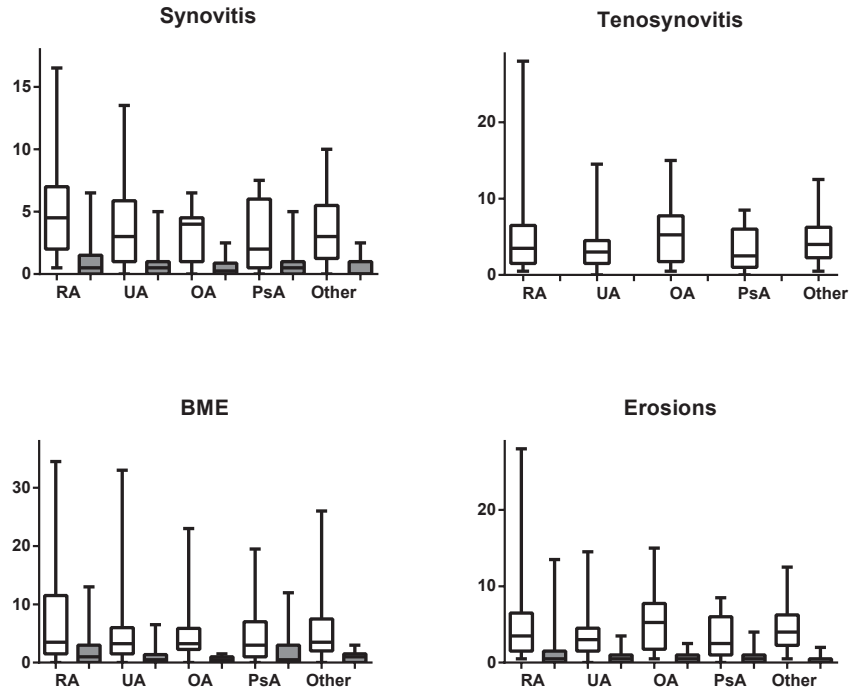
1. Van Nies JA, Krabben A, Schoones JW, et al. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis* 2013.
2. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003;48:1814–1827.
3. Colebatch AN, Edwards CJ, Ostergaard M, et al. EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis. *Ann Rheum Dis* 2013.
4. Suter LG, Fraenkel L, Braithwaite RS. Role of magnetic resonance imaging in the diagnosis and prognosis of rheumatoid arthritis. *Arthritis Care Res* 2011;63:675–688.
5. Tamai M, Kawakami A, Uetani M, et al. A prediction rule for disease outcome in patients with undifferentiated arthritis using magnetic resonance imaging of the wrists and finger joints and serologic autoantibodies. *Arthritis Rheum* 2009;61:772–778.
6. De Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatol Oxf Engl* 2011;50:93–100.
7. Haavardsholm EA, Østergaard M, Ejbjerg BJ, et al. Introduction of a novel magnetic resonance imaging tenosynovitis score for rheumatoid arthritis: reliability in a multireader longitudinal study. *Ann Rheum Dis* 2007;66:1216–1220.
8. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J Biom Z* 2005;47:458–472.
9. Calisir C, Murat Aynaci AI, Korkmaz C. The accuracy of magnetic resonance imaging of the hands and feet in the diagnosis of early rheumatoid arthritis. *Jt Bone Spine Rev Rheum* 2007;74:362–367.
10. Ostendorf B, Scherer A, Mödder U, et al. Diagnostic value of magnetic resonance imaging of the forefeet in early rheumatoid arthritis when findings on imaging of the metacarpophalangeal joints of the hands remain normal. *Arthritis Rheum* 2004;50:2094–2102.
11. Burgers LE, van Nies JA, Ho LY, et al. Long-term outcome of Rheumatoid Arthritis defined according to the 2010-classification criteria. *Ann Rheum Dis* 2013.
12. Skogh T. Does a positive anti-CCP test identify a distinct arthritis entity? *Arthritis Res Ther* 2005;7:230–232.
13. Tamai M, Kawakami A, Uetani M, et al. The presence of anti-cyclic citrullinated peptide antibody is associated with magnetic resonance imaging detection of bone marrow oedema in early stage rheumatoid arthritis. *Ann Rheum Dis* 2006;65:133–134.
14. Krabben A, Stomp W, van der Heijde DM, et al. MRI of hand and foot joints of patients with anticitrullinated peptide antibody positive arthralgia without clinical arthritis. *Ann Rheum Dis* 2013;72:1540–1544.
15. Harre U, Georgess D, Bang H, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012;122:1791–1802.



Supplementary Figure 1: RAMRIS-scores for the different MRI features for RA patients according to 1987 and 2010 criteria
Horizontal lines represent median values.



Supplementary Figure 2: RAMRIS-scores for the different MRI features for RA patients fulfilling both the 1987 and 2010 criteria and one of these two sets of criteria.
Horizontal lines represent median values. 1987-/2010+ versus 1987+/2010+ patients: synovitis p=0.029. All other combinations p>0.05.



Supplementary Figure 3: RAMRIS-scores for the different MRI features per group of diagnoses, separated for hand and foot joints

Box and whisker plots showing median, interquartile and range of scores separately for the hand (wrist and MCP joints combined, white) and forefoot (grey). Tenosynovitis was only assessed in the hand.

PART 4

MRI scan protocol revisited

13

Aiming for a shorter rheumatoid arthritis MRI protocol: can contrast-enhanced MRI replace T2 for the detection of bone marrow oedema?

W. Stomp, A. Krabben, D. van der Heijde, T. W. J. Huizinga, J. L. Bloem, A. H. M. van der Helm-van Mil, M. Reijnierse

European Radiology. 2014 October;24 (10):2614-22.

ABSTRACT

Objective

To determine whether T1 post-Gadolinium chelate images (T1Gd) can replace T2-weighted images (T2) for evaluating bone marrow edema (BME), thereby allowing shortening the Magnetic Resonance Imaging (MRI) protocol in rheumatoid arthritis (RA).

Methods

In 179 early arthritis patients and 43 advanced RA patients wrist and metacarpophalangeal joints were imaged on a 1.5T extremity MRI system with a standard protocol (coronal T1-, T2 fat saturated and coronal and axial T1 fat saturated after Gd). BME was scored according to OMERACT RAMRIS by two observers with and without T2-images available. Agreement was assessed using ICCs for semi-quantitative scores and test characteristics with T2 images as reference.

Results

4,048 and 989 bones were assessed in 179 early arthritis and 43 advanced RA patients respectively. Agreement between scores based on T2 and T1Gd images was excellent (intra class correlation coefficients (ICC) 0.80-0.99). On bone level sensitivity and specificity of BME on T1Gd compared to T2 were high for both patient groups and both readers (all ≥80%).

Conclusion

T1Gd and T2 images are equally suitable for evaluating BME. Because contrast is usually administered to assess (teno)synovitis, a short MRI protocol of T1 and T1Gd is sufficient in RA.

INTRODUCTION

Bone marrow edema (BME) is one of the main features of rheumatoid arthritis (RA) that can be seen on magnetic resonance imaging (MRI).[1,2] BME is an independent predictor of subsequent radiographic progression in early RA.[3-5] The OMERACT Rheumatoid Arthritis MRI Scoring system (RAMRIS) is a standardized scoring system for the assessment of synovitis, bone marrow edema and erosions on MRI in RA. It defines BME as a lesion within trabecular bone, with ill-defined margins and signal characteristics consistent with increased water content, i.e. high signal on T2-fatsat and STIR images and low signal on T1.[6]

RAMRIS recommends that imaging includes T1-weighted sequences before and following contrast agent administration, and T2-weighted images with frequency selective fat saturation (T2) or short tau inversion recovery (STIR) if frequency selective fat suppression is not available. T1-weighted images are primarily used to assess erosions and synovitis, whereas T2 images are used to evaluate BME. However, T1-weighted sequences with gadolinium-chelate (Gd) contrast-enhancement and fat-suppression (T1Gd) produce images very similar to T2 images (Figure 1). Although the RAMRIS core set of MRI acquisitions does not describe the use of fat-suppression for the post-contrast T1, in practice this is routinely used to enhance visibility of enhancement and to differentiate enhancement from fatty tissue on fast spin echo sequences, which exhibit a high signal of fat secondary to J-coupling.[7] Previous studies performed in the knee, ankle and foot have shown that T2- fat-suppressed or STIR images and T1Gd MRI images demonstrate almost identical imaging patterns for BME.[8,9]

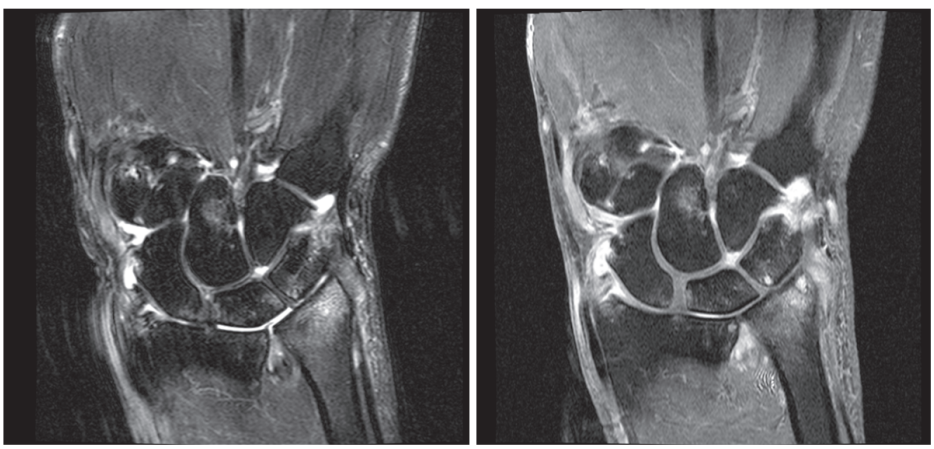


Figure 1: Typical appearance of BME on both sequences
Coronal T2 weighted fat-suppressed (a) and contrast enhanced T1-weighted fat-suppressed images (b) of the wrist in the same patient showing an almost identical pattern of BME., The majority of high intra-articular signal on T2 (a) enhances (b) consistent with synovitis; a small amount of fluid is present in the radiocarpal joint.

Scanning of unilateral hand, wrist and foot joints according to RAMRIS protocol takes >60 minutes. T1Gd images form an essential part of the protocol as they are essential to assess synovitis and tenosynovitis.[10-12] If T1Gd images could also be used to score BME in small hand and foot joints, valuable imaging time might be saved by leaving out T2 sequences which account for approximately 20-25% of the examination time. A shorter protocol would reduce costs and the discomfort of patients and increase the accessibility of MR. Therefore we aimed to evaluate whether T1Gd images can replace T2 images for scoring of BME, without loss of information. We studied patients with early arthritis and active advanced RA to ensure that the results observed were not dependent on the severity of the BME lesions.

MATERIALS AND METHODS

Patients

Two groups of patients were studied. The first group consisted of 179 patients presenting with early arthritis to the rheumatologic outpatient clinic of the Leiden University Medical Center, the Netherlands. Forty-three (24%) of these patients fulfilled the ACR87 criteria for RA, the others were diagnosed as undifferentiated arthritis (n=88, 49%), inflammatory osteoarthritis (n=12, 7%), psoriatic arthritis (n=15, 8%) and other rheumatic diagnoses (n=21, 12%). The median age was 57 years (interquartile range (IQR) 45-66) and 99 (55%) were female. Forty-five (25%) patients were anti-citrullinated peptide antibody (ACPA) positive. The median disease activity score (DAS44) score was 2.5 (IQR1.9-3.0).[13]

The second group consisted of 43 advanced RA patients with RA according to ACR87 criteria with a DAS44 higher than 2.4. Their disease was active despite treatment with conventional disease modifying anti-rheumatic drugs including maximal tolerable doses of methotrexate. The median age was 57 (IQR 51-62) and 31 (72%) were female. All advanced RA patients were positive for ACPA.

MRI

Examinations were performed on a MSK Extreme 1.5 Tesla extremity scanner (GE, Wisconsin, USA). The complete recommended RAMRIS imaging set was acquired for the wrist and MCP. Joints were scanned at the most painful, or if indifferent, dominant side.

The following sequences were acquired: T1-weighted fast spin echo (FSE) sequence in the coronal plane (repetition time (TR)/echo time (TE) 650/17 ms; acquisition matrix 388 × 288; echo train length (ETL) 2); T2-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 3000/61.8 ms; acquisition matrix, 300 x 224, ETL 7). All sequences were acquired separately for the wrist and MCP joints, limited by the field of view of 100mm. Gd-chelate contrast agent (gadoteric acid, Guerbet, Paris, France)

was administered intravenously at a standard dose of 0.1 mmol/kg. After injection, T1-weighted FSE sequence with frequency selective fat saturation (T1Gd) in the coronal plane was performed (TR/TE 650/17 ms, acquisition matrix 364 × 224, ETL 2) and a T1Gd in the axial plane (TR/TE 570/7 ms; acquisition matrix 320 × 192; ETL 2). Again all sequences were acquired for both wrist and MCP joints. Field-of-view was 100mm for all sequences. Coronal sequences had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm while the axial sequence had 20 slices with a slice thickness of 3mm and a slice gap of 0.3mm.

Image assessment

BME was defined as a lesion within the trabecular bone with ill-defined margins and high signal intensity on the T2 or T1Gd images. It was scored on a 0-3 scale for each bone according to OMERACT RAMRIS score by two trained readers independently.[6] Scores were defined as 0=0%; 1=1-33%; 2=34-66%, 3=67-100% of bone affected up to 1 cm from the joint. In case of erosions or cysts, this percentage corresponds to the part of the remaining bone affected. Scoring was performed once on T2 images and a second time on T1Gd at least two weeks apart and with images anonymized and their order randomized between sessions. 14% of the early arthritis MRI imaging sets were read twice to determine the intra-reader reliability of scoring.

Image quality was assessed by one reader separately for the T2 weighted and T1Gd images on a 0-4 scale. Scores assigned were 0 completely not assessable, 1 partly not assessable, 2 poor, 3 adequate and 4 good image quality, taking into account motion artifacts, signal and contrast to noise ratios and other factors influencing image quality.

Statistics

Image quality scores were compared by Wilcoxon signed ranks test. Differences in BME scores were compared using a paired Student's t-test and the correlation between the sequences was assessed using a Pearson correlation coefficient. Because it is desirable for scores on both sequences to have not only good correlation, but also to yield similarity in absolute BME scores, agreement between scores was assessed using intra class correlation coefficients (ICC) for absolute agreement. Agreement was also visualized by means of Bland-Altman plots in order to detect systemic biases.

Assessments for the presence or absence of BME were made both on individual bone level and at patient level (BME present in any joint). At bone level, a score of ≥1 in any individual bone was considered positive. Similarly, at patient level, a total score of ≥1 was considered positive. Sensitivity and specificity of T1Gd were determined with presence of BME on T2 as the reference standard. When values were missing on either T1Gd or T2, these bones were discarded from both assessments. The data of both readers were assessed separately, to validate that results obtained were not based on one single reader. We decided that, in

order to be able to replace T2 with T1Gd, acceptable levels of agreement were: Pearson correlation coefficient and ICC of ≥0.80 and sensitivity ≥80% as assessed by two readers.

RESULTS

In the early arthritis group three patients did not receive Gd due to the presence of a low estimated glomerular filtration rate or refusal of the patient. Thus in 176 early arthritis and 43 advanced RA patients, 4048 and 989 bones were evaluated. The intra-reader ICCs for BME on T2 were 0.96 for reader 1 and 0.72 for reader 2. Missing values were present for reader 1 and 2 in 22 and 39 (0.5-1.0%) bones on T2 and 20 and 21 (0.5-0.5%) bones on T1Gd in early arthritis and 24 and 36 (2.4-3.6%) bones on T2 and 21 and 23 (2.1-2.3%) bones on T1Gd in advanced RA patients.

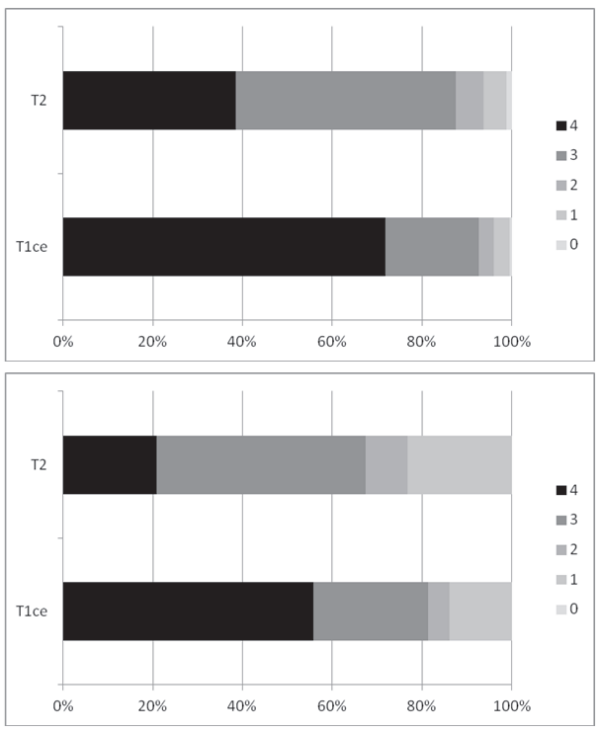


Figure 2: Image quality
Image quality for both sequences in early arthritis (a) and advanced RA (b). Scores: 4 good image quality, 3 adequate image quality, 2 poor image quality, 1 partly not assessable, 0 completely not assessable.

Image quality

In early arthritis patients, BME could be assessed in 174 patients on T2 (98.9%) and 175 patients on T1Gd (99.4%). Images were partly not assessable (image quality score 1) in nine patients on T2 and six patients on T1Gd. In advanced RA BME could be assessed in all 43 patients, although images were partly not assessable in ten patients on T2 and six patients on T1Gd. In both patient groups incomplete fat suppression was the reason for being partly not assessable. Completely not assessable scans were very rare (less than 2%, see Figure 2) and were all caused by excessive motion artifacts. Overall image quality was rated better on T1Gd than on T2 images. The median image quality score was four on T1Gd and three on T2 in both early arthritis and advanced RA (both $p<0.001$). In case of partly not assessable images (score of 1), the parts of the image that were assessable were still used for all further analyses.

Table 1. Presence and scores of BME and measures of correlation and test characteristics at patient level

	Early arthritis (n=176)		*Advanced RA (n=43)*	
	Reader 1	Reader 2	Reader 1	Reader 2
	Prevalence			
BME prevalence, T2	143 (81.3%)	152 (86.4%)	41 (95.3%)	40 (93.0%)
BME prevalence, T1Gd	146 (83.0%)	159 (90.3%)	40 (93.0%)	43 (100%)
Concordance of T2 and T1Gd for presence of BME	165 (93.8%)	155 (88.1%)	40 (93.0%)	40 (93.0%)
Sensitivity of T1Gd (95% CI)	97.2% (92.5-99.1%)	95.4% (90.4-98.0%)	95.1% (82.2-99.2%)	100.0% (89.0-100%)
Specificity of T1Gd (95% CI)	78.8% (60.6-90.4%)	41.7% (22.8-63.1%)	50.0% (2.7-97.3%)	0.0% (0.0-69.0%)
	Scores			
Median score T2 (IQR)	3 (1-6)	3.5 (2-9)	8 (3-20)	6 (2-20)
Median score T1Gd (IQR)	3 (1-6)	5 (2-9)	7 (3-19)	8 (5-17)
ICC between T2 and T1Gd (95% CI)	0.99 (0.98-0.99)	0.87 (0.82-0.90)	0.99 (0.98-0.99)	0.93 (0.86-0.96)
- ICC, Wrist only (95% CI)	0.98 (0.97-0.98)	0.86 (0.81-0.90)	0.99 (0.99-1.00)	0.94 (0.88-0.96)
- ICC, Metacarpals only (95% CI)	0.98 (0.97-0.98)	0.85 (0.80-0.88)	0.90 (0.81-0.94)	0.80 (0.66-0.89)

Paired t-test was applied to test for differences in median scores between T2 and T1gd in early arthritis: p=0.73 (reader 1) and 0.27 (reader 2); and in advanced RA: p= 0.91 (reader 1) and 0.52 (reader 2). Presence of BME defined as a score of ≥1. T2 sequence is the reference standard for sensitivity and specificity. Intra reader ICC's for agreement between scores based on T2 and on T1Gd.

Prevalence of BME in RA

In early arthritis patients, BME (a score of ≥1) was present in 143 (81.3%) and 152 (86.4%) patients on T2 images and 146 (83.0%) and 159 (90.3%) patients on T1Gd images for reader 1 and 2 respectively (Table 1). In advanced RA patients, BME was present in 41 (95.3%) and 40 (93.0%) patients on T2 images and 40 (93.0%) and 43 (100.0%) patients on T1Gd images for reader 1 and 2 respectively. Thus BME was present in the majority of patients in both groups. BME scores were higher in advanced than in early RA (Table 1).

When evaluating the presence of BME in individual bones, in early arthritis BME was present in 677 (16.7%) and 921 (23.1%) bones on T2 images and 683 (17.0%) and 1023 (25.6%) bones on T1Gd for reader 1 and 2 respectively (Table 2). Likewise, in advanced RA patients, BME was present in 311 (32.7%) and 299 (31.3 %) bones on T2 images and 307 (32.3%) and 372 (39.0%) bones on T1Gd images for reader 1 and 2.

Table 2. Presence of BME and test characteristics in individual bones

	Early arthritis (n=176)		*Advanced RA (n=43)*	
	Reader 1	Reader 2	Reader 1	Reader 2
	Individual bone level			
Total bones	4048		989	
Missing values, T2	22 (0.5%)	39 (1.0%)	36 (3.6%)	24 (2.4%)
Missing values, T1Gd	21 (0.5%)	20 (0.5%)	23 (2.3%)	21 (2.1%)
Assessed bones	4017	3995	950	955
BME prevalence, T2	677 (16.7%)	921 (23.1%)	311 (32.7%)	299 (31.3%)
BME prevalence, T1Gd	683 (17.0%)	1023 (25.6%)	307 (32.3%)	372 (39.0%)
Concordance of T2 and T1Gd for presence of BME	3839 (95.6%)	3523 (88.2%)	894 (94.1%)	814 (85.2%)
Discordance >1 point	5 (0.1%)	21 (0.5%)	21 (2.2%)	10 (1.0%)
- Sensitivity of T1Gd	87.3% (84.5-89.7%)	80.0% (77.3-82.5%)	90.4% (86.4-93.3%)	88.6% (84.3%-91.9%)
- Specificity of T1Gd	97.2% (96.6-97.8%)	90.7% (89.6-91.7%)	95.9% 94.0-97.3%)	83.7% (80.6-86.4%)

BME=bone marrow edema. Presence of BME defined as a score of ≥1. T2 sequence is the reference standard for sensitivity and specificity. Intra reader ICC's for agreement between scores based on T2 and on T1Gd.

Comparison of BME evaluated on a semi-quantitative scale

First we compared the BME scores between both sequences (Table 1). In early arthritis patients, median scores were 3 on T2 and 3.5 on T1Gd for reader 1 (p=0.73) and 3.5 on T2 and 5 on T1Gd images for reader 2 (p=0.27). In advanced RA patients, median scores were 8 on T2 and 7 on T1Gd for reader 1 (p=0.91) and 6 on T2 and 8 on T1Gd images for reader 2 (p=0.52).

Figure 3 shows scores based on T1Gd plotted against scores on T2, showing a high degree of correlation between scores on T2 and T1Gd (early arthritis r=0.99 and 0.87 for reader 1 and 2, and advanced RA r=0.99 and 0.94).

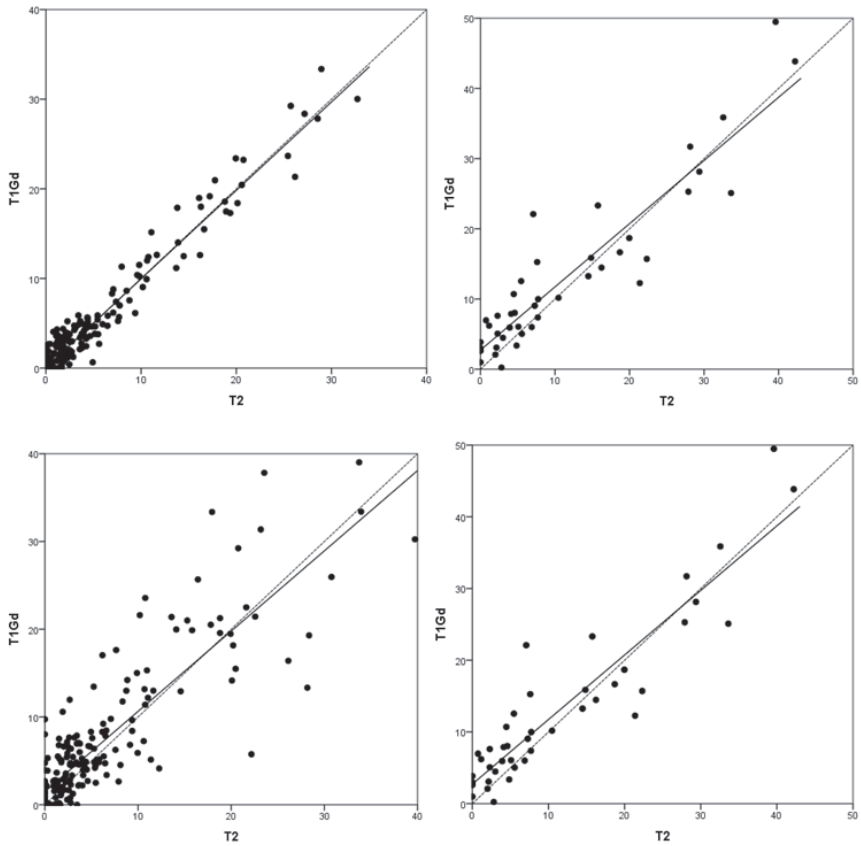


Figure 3: Correlation between total BME scores scored on T1Gd and T2 sequences.
Scores for observer 1 in early arthritis (a) and advanced RA (b) and for observer 2 in early arthritis (c) and advanced RA (d). Scores on T2 on the horizontal and scores on T1Gd on the vertical axis. Solid line: linear regression line; dashed line indicates the best possible (1:1) correlation.

Subsequently the level of agreement of scores obtained on T2 and T1gd were evaluated using ICCs. In early arthritis, the ICC's for both readers were 0.87 and 0.99; in advanced RA these were 0.99 and 0.93 (Table 1). Bland-Altman plots (Figure 4) revealed little systematic differences (reader 2 had slightly higher scores on T1Gd) and acceptable 95% limits of agreement for the differences in scores.

Comparison of the presence or absence of BME

For clinical application, determining the presence or absence of BME might be more important than the score on a semi-quantitative scale. We assessed test characteristics of T1Gd with T2-images as the reference standard with a score of ≥1 as cut-off for positivity. Analyses were done at the individual bone level and showed that sensitivity was ≥80%

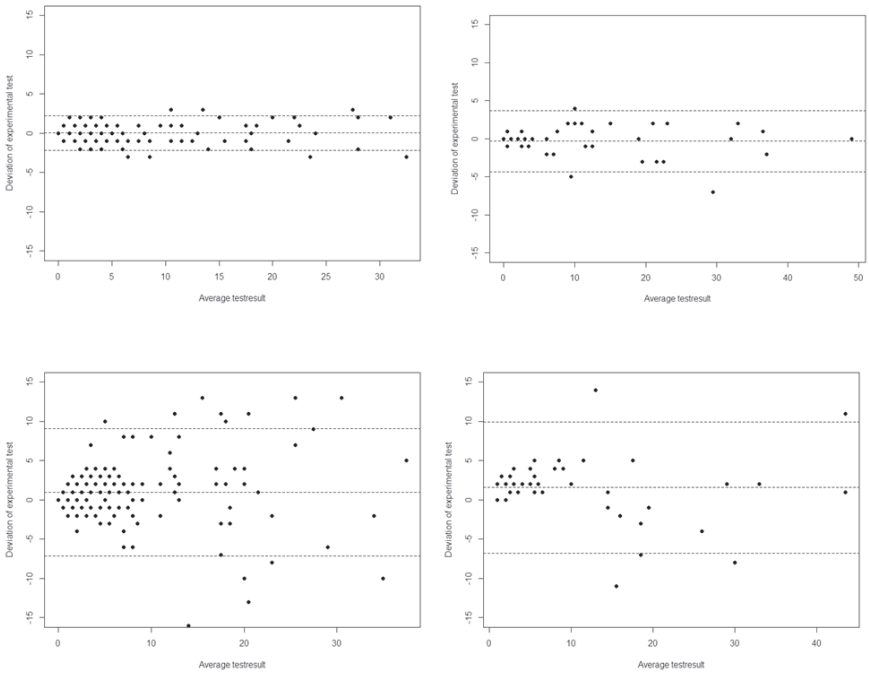


Figure 4: Bland-Altman plots of total patient BME scores on T1Gd and T2 sequences
Bland-Altman plots for observer 1 in early arthritis (a) and advanced RA (b) and for observer 2 in early arthritis (c) and advanced RA (d). The difference (T1Gd-T2) between paired measurements are plotted against the mean of the two measurements. The middle line in each graph shows the bias between the two measurement methods. The observation that the line is located around 0 indicates that systematic bias was low; although reader 2 achieved slightly higher scores on T1Gd and showed some heteroscedasticity on both sequences (variance increases with an increase in score), which was also present to a lesser extent in advanced RA for reader 1. The dashed lines show the ± 95% limits of agreement.

and specificity ≥ 83% (Table 2) in the various tested combinations. When evaluating the test characteristics of the presence of BME at the patient level, also a high sensitivity was observed, ≥ 95% for both readers. However, the specificity was low with a broad 95%CI, which is partly explained by the low number of patients without BME (17-33 early arthritis patients and 0-3 advanced RA patients depending on reader and sequence). A high level of agreement between T1Gd and T2 was also illustrated by the high concordance in bones that were scored as having BME and the low frequency of discordance of more than one point (≤0.5% in early RA and ≤2.2% in advanced RA (Table 2).

Reliability analysis (inter-reader agreement)

Finally we also assessed the reliability of scoring between both readers when evaluating T2 or T1Gd. The ICCs were high for both sequences (all ≥0.83, Table 3), indicating good inter-reader agreement under all investigated conditions.

Table 3. Interreader ICCs for BME scores at patient and bone level

	Patient		*Bone*	
	Early arthritis	*Advanced RA*	*Early arthritis*	*Advanced RA*
T2 BME	0.86 (0.80-0.90)	0.91 (0.84-0.95)	0.77 (0.75-0.79)	0.86 (0.84-0.88)
- Wrist	0.90 (0.86-0.93)	0.96 (0.92-0.98)	0.79 (0.77-0.80)	0.87 (0.85-0.89)
- Metacarpals	0.75 (0.67-0.81)	0.67 (0.45-0.81)	0.71 (0.68-0.74)	0.81 (0.75-0.86)
T1Gd BME	0.88 (0.69-0.94)	0.93 (0.87-0.96)	0.80 (0.77-0.82)	0.85 (0.83-0.86)
- Wrist	0.89 (0.72-0.95)	0.95 (0.90-0.97)	0.80 (0.76-0.83)	0.84 (0.82-0.87)
- Metacarpals	0.83 (0.77-0.88)	0.66 (0.46-0.80)	0.78 (0.75-0.80)	0.85 (0.81-0.87)

Inter reader ICCs for total BME score per patient and for individual bone scores between the two readers, by patient group and imaging sequence used.

DISCUSSION

T1Gd images have almost the same yield as T2 images in displaying BME. Our results show that BME is equally well assessed on either sequence. Thus, when coronal fat-suppressed T1 weighted images after Gd-chelate contrast administration are routinely obtained as part of the imaging protocol, as is the case within the OMERACT RAMRIS core imaging set, T2 or STIR images are redundant and can be eliminated from the imaging protocol, reducing total imaging time by approximately 20-25%.

Historically RA has been considered a disease that mainly involved the synovium, with erosions caused by pannus invasion. It was only after the introduction of MRI that it was observed that inflammatory processes take place within the bone, as reflected by BME-like abnormalities on MR. Although for some years it remained unclear what the significance of BME was, it has now been shown that BME detected by MRI reflects the formation of inflammatory infiltrates in the bone marrow in RA.[14] Histological examination of BME reveals a number of cell types, including macrophages, plasma cells, CD8+ T cells and B cells.[15] Stressing the importance of this process is the finding that BME is the strongest imaging predictor of erosive progression that has been identified to date.[1]

On MRI BME can be observed due to the focally increased water content in the bone marrow, partly or entirely replacing normal bone marrow fat. Signal intensity is low on T1 sequences and high on T2 or STIR sequences. BME also enhances with intravenous Gd.[16] The appearance of bone marrow on T1Gd images is very similar to T2 or STIR images before Gd contrast administration.[8,9]

Previously it has been shown in the knee, ankle and foot of patients with non-rheumatic diseases that T2 or STIR images and T1Gd images are equally suitable to assess BME and other bone marrow abnormalities.[8,9] In early RA, findings from Tamai et al. suggest that T1Gd images visualize bone marrow edema with high specificity compared to T2.[11] Our

study is, as far as we know, the first focussing specifically on the sequences required to image BME in RA.

The standardized use of fat suppression on the T1Gd sequence aids in identifying enhancing BME in the fatty bone marrow. Protons in both fatty and aqueous environment have high signal intensity on T2FSE and can be differentiated by using fat saturation, identifying the water as high signal intensity (Figure 1a). Nowadays fat saturation in combination with T2FSE is routinely included in musculoskeletal imaging protocols. The same additional value of fat saturation is used in the contrast enhanced T1FSE sequences, facilitating the depicting of Gd enhancement (high on T1) in the high signal intensity of fatty marrow (Figure 1b). Our results show that detection of BME on T1Gd is similar to that on T2 images. In addition, better image quality favors the T1Gd sequence. One limitation of using only T1Gd sequence is that small effusions, bright on T2 but not enhancing on T1Gd, may be harder to detect. Three patients did not receive Gd and thus these could be considered failures for the T1Gd images; however whenever contrast administration is not feasible, T2 can always be used as a fallback option.

Within the imaging protocol for arthritis as used in our hospital, the T2 sequence takes approximately four minutes out of twenty for the complete protocol for one joint area. Thus eliminating the T2 sequence from the imaging protocol results in a 20% reduction of imaging time. Especially when imaging multiple joint areas in one session, shortening of the imaging time in combination with more robust sequences decreases the chance of unsuccessful MRI examinations.

A limitation of our study was that we performed semi-quantitative measurements rather than quantitative measurements of BME volume. However this reflects current research practice where RAMRIS is the predominantly used method of semi-quantification. Moreover, previous studies have shown the measured volume of BME to be almost identical regardless of imaging sequence.[8,9] Also, our method requires the administration of Gd, however this is usually not an issue, as this is needed for the assessment of synovitis and tenosynovitis.

Strengths of our study include the large number of patients studied, the inclusion of both early arthritis and advanced rheumatoid arthritis patients and the data having evaluated by two readers. The consistency in the findings between readers and between patients with different severity of BME lesions and different severity of RA support the validity of our results.

In conclusion, T1Gd and T2 images are equally suitable for scoring BME in early arthritis patients and advanced RA. For RAMRIS scoring, a short protocol of T1 and T1Gd is sufficient.

REFERENCE LIST

1. McQueen. FM Bone marrow edema and osteitis in rheumatoid arthritis: the imaging perspective. *Arthritis Res Ther* 2012;14:224.
2. Bugatti S, Manzo A, Caporali R, et al. Inflammatory lesions in the bone marrow of rheumatoid arthritis patients: a morphological perspective. *Arthritis Res Ther* 2012;14:229.
3. Hetland ML, Ejbjerg B, Hørslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009;68:384–390.
4. Bøyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011;70:428–433.
5. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003;48:1814–1827.
6. Østergaard M, Peterfy C, Conaghan P, et al. OMERACT Rheumatoid Arthritis Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. *J Rheumatol* 2003;30:1385–1386.
7. Henkelman RM, Hardy PA, Bishop JE, et al. Why fat is bright in RARE and fast spin-echo imaging. *J Magn Reson Imaging* 1992;2:533–540.
8. Schmid MR, Hodler J, Vienne P, et al. Bone marrow abnormalities of foot and ankle: STIR versus T1-weighted contrast-enhanced fat-suppressed spin-echo MR imaging. *Radiology* 2002;224:463–469.
9. Mayerhoefer ME, Breitenseher MJ, Kramer J, et al. STIR vs. T1-weighted fat-suppressed gadolinium-enhanced MRI of bone marrow edema of the knee: computer-assisted quantitative comparison and influence of injected contrast media volume and acquisition parameters. *J Magn Reson Imaging* 2005;22:788–793.
10. Ostergaard M, Conaghan PG, O'Connor P, et al. Reducing invasiveness, duration, and cost of magnetic resonance imaging in rheumatoid arthritis by omitting intravenous contrast injection — Does it change the assessment of inflammatory and destructive joint changes by the OMERACT RAMRIS? *J Rheumatol* 2009;36:1806–1810.
11. Tamai M, Kawakami A, Uetani M, et al. Magnetic resonance imaging (MRI) detection of synovitis and bone lesions of the wrists and finger joints in early-stage rheumatoid arthritis: comparison of the accuracy of plain MRI-based findings and gadolinium-diethylenetriamine pentaacetic acid-enhanced MRI-based findings. *Mod Rheumatol* 2012;22:654–658.
12. Tehranzadeh J, Ashikyan O, Anavim A, et al. Enhanced MR imaging of tenosynovitis of hand and wrist in inflammatory arthritis. *Skeletal Radiol* 2006;35:814–822.
13. Van der Heijde DM, van 't Hof M, van Riel PL, et al. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* 1993;20:579–581.
14. Jimenez-Boj E, Nöbauer-Huhmann I, Hanslik-Schnabel B, et al. Bone erosions and bone marrow edema as defined by magnetic resonance imaging reflect true bone marrow inflammation in rheumatoid arthritis. *Arthritis Rheum* 2007;56:1118–1124.
15. Dalbeth N, Smith T, Gray S, et al. Cellular characterisation of magnetic resonance imaging bone oedema in rheumatoid arthritis; implications for pathogenesis of erosive disease. *Ann Rheum Dis* 2009;68:279–282.
16. Hodgson R, Grainger A, O'Connor P, et al. Dynamic contrast enhanced MRI of bone marrow oedema in rheumatoid arthritis. *Ann Rheum Dis* 2008;67:270–272.

14

Aiming for a shorter scanning protocol in early arthritis MRI: can gadolinium contrast administration be eliminated?

W. Stomp, A. Krabben, D. van der Heijde, T. W. J. Huizinga, J. L. Bloem, M. Østergaard, A. H. M. van der Helm-van Mil, M. Reijnierse

European Radiology, Submitted.

ABSTRACT

Objective

Magnetic Resonance Imaging (MRI) is increasingly used in Rheumatoid Arthritis (RA) research to depict local inflammation. According to the RAMRIS-protocol intravenous (IV) contrast is administered to assess synovitis and tenosynovitis. We studied whether IV-contrast can be eliminated, decreasing imaging time, cost and invasiveness.

Methods

Wrist MRIs of 93 early arthritis patients were evaluated by two readers for synovitis of the radio-ulnar, radio-carpal and intercarpal joints, according to RAMRIS, and for tenosynovitis in ten compartments. Scores of MR-images without IV-contrast-enhancement were compared to scores obtained when evaluating all, including contrast-enhanced, MRI-images as reference. Subsequently a literature review and pooled analysis of data from the present and two previous studies were performed.

Results

At individual joint/tendon level, sensitivity to detect synovitis without contrast was 91% and 72%, respectively, for the two readers and specificity 51% and 81%, with contrast-enhanced images as reference standard. For tenosynovitis sensitivity was 67% and 54%, respectively and specificity 87% and 91%. Pooled data analysis revealed an overall sensitivity of 81% and specificity of 50% for evaluation of synovitis. Variations in tenosynovitis scoring systems hindered pooled analyses.

Conclusion

Eliminating IV-contrast decreased specificity for synovitis and sensitivity for tenosynovitis, indicating that IV-contrast remains essential for an optimal assessment.

INTRODUCTION

Magnetic Resonance Imaging (MRI) is increasingly used in research of Rheumatoid Arthritis (RA). MRI has high sensitivity to depict local inflammation in the form of synovitis, tenosynovitis and bone marrow edema.[1] The scanning protocol is standardized in the OMERACT Rheumatoid Arthritis MRI scoring (RAMRIS) method.[2] OMERACT recommended MRI sequences include non-contrast enhanced T2 weighted fat saturated images (T2) or short tau inversion recovery (STIR) images to evaluate bone marrow edema,[2] whereas pre- and post-gadolinium contrast T1-weighted images (T1Gd) have been recommended for evaluation of synovitis and tenosynovitis.[2–4]

The use of intravenous (IV) gadolinium contrast has drawbacks; it is an invasive procedure, it is costly and it prolongs the imaging required time. Synovitis and tenosynovitis normally exhibit high signal intensity both on T2 and T1Gd images (illustrated in Supplementary Figure 1). We therefore hypothesized that it is possible to evaluate synovitis and tenosynovitis on T2 instead of T1Gd. When IV-contrast administration could be eliminated this would make MRI more patient-friendly and would increase accessibility.

The objective of this study was to determine whether IV contrast administration could be eliminated from the scanning protocol when assessing synovitis and tenosynovitis. This was achieved by a study of 93 early arthritis patients, a literature review and an analysis of pooled data from the above-mentioned material and two previous studies.

MATERIALS AND METHODS

Patients

Between July 2011 and April 2012 MR imaging was performed in 93 early arthritis patients at the first visit of the Leiden Early Arthritis Clinic; for further reading on the Leiden EAC see.[5] These patients were part of a larger group in whom MRI was performed; the current study concerns a subgroup in which an extra axial T2-weighted sequence of the wrist was obtained. All patients provided informed consent and the study was approved by the institutional review board.

MRI

MRI of the wrist was performed at the most painful or the dominant side in case of equally severe symptoms. Coronal T1-weighted images and coronal and axial T2-weighted images with fat suppression were acquired. After IV contrast injection, coronal and axial T1-weighted images with fat suppression were acquired (full MRI protocol provided in the Supplementary Methods).

Anonymized datasets were scored twice by two experienced readers (WS and AK), using all acquired images (Gdset), and using only unenhanced images (T2set). The order of examinations was randomized and there was an interval of at least two months between assessments. Images were scored for synovitis according to RAMRIS on a 0-3 scale for the radio-ulnar, radio-carpal and the combined intercarpal and carpometacarpophalangeal joints.[2] Tenosynovitis was evaluated in 10 tendons/compartments on a 0-3 scale as described by Haavardsholm et al.[4]

Reference standard and statistics

Gadolinium enhanced image scores were the reference standard. Comparisons were made for the two readers independently and for the agreement between readers. To determine whether the same absolute scores were obtained by both methods, scores were compared with weighted kappa statistic and intra class correlation coefficient (ICC) for absolute agreement. Furthermore, the sensitivity and specificity were calculated at both joint/tendon level and at patient level, with scores ≥1 considered positive at both joint/tendon and patient level.

Literature review and pooled data analysis

Available literature up to November 2013 was searched; central terms in our search were 'arthritis', 'synovitis', 'tenosynovitis', 'gadolinium contrast' and 'MRI' (full search strategy provided in the Supplementary Methods). Studies comparing findings on MRI for synovitis and tenosynovitis with and without IV-contrast were reviewed. For synovitis we performed a pooled data analysis; raw data were obtained from the literature[6] or obtained via personal communication[7] and combined to determine overall test characteristics. For tenosynovitis, due to different scoring systems used we could not perform a pooled data analysis.

RESULTS

Data from 92 patients were analyzed, as one MRI was excluded because of severe artifacts caused by a metallic foreign body. Patient characteristics are listed in Supplementary table 1. Based on reader 1 scores for Gdset (the reference standard) MRI synovitis was present in 162 joints (59%) and 81 patients (88%); tenosynovitis was present in 153 tendon compartments (17%) and 52 patients (57%).

Agreement for total synovitis and tenosynovitis scores

For total scores within each patient, Bland-Altman plots showed acceptable levels of agreement (Figure 1). For tenosynovitis there was a tendency towards more variation with higher scores (heteroscedasticity) especially for reader 2. There was little systematic bias for both

readers between the sets with and without contrast. ICCs between the T2set (without contrast) and Gdset (with gadolinium contrast) images were 0.75 (95%CI 0.54-0.86) and 0.82 (95%CI 0.74-0.88) for synovitis for the two readers, respectively and 0.72 (95%CI 0.60-0.81) and 0.57 (95%CI 0.42-0.70) for tenosynovitis, indicating moderate to good agreement for total synovitis and tenosynovitis scores.

Test characteristics on patient level

When evaluating the presence of synovitis at patient level without gadolinium contrast (the T2set), the sensitivity was 96% and 78%, respectively, for the two readers and the specificity was 36% and 71%. When tenosynovitis was assessed using the T2set the sensitivity was 89% and 71% and the specificity 40% and 68% (Table 1).

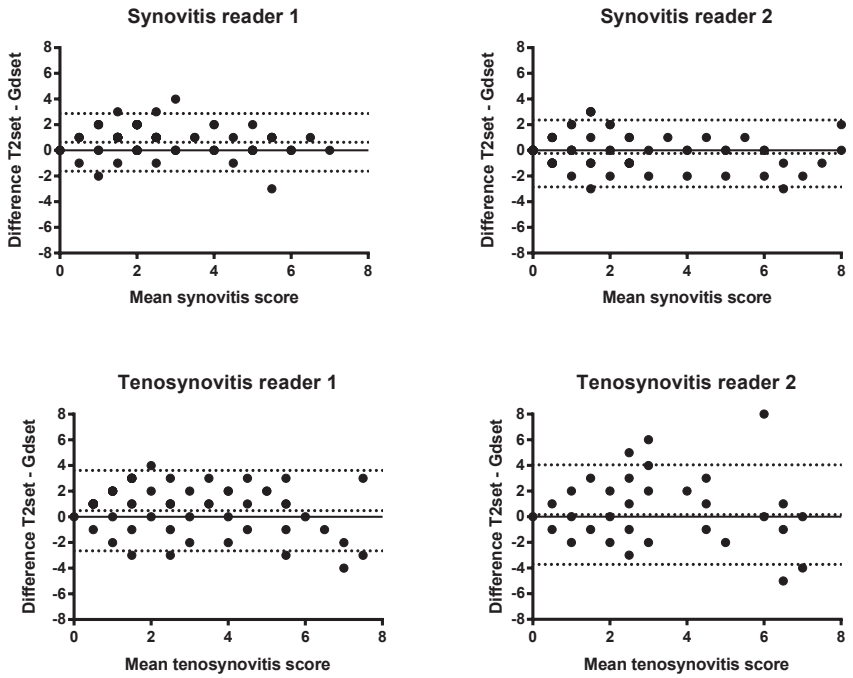


Figure 1: Bland-Altman plots of assessment of synovitis and tenosynovitis with and without gadolinium enhancement

Bland-Altman plots for total scores for synovitis (upper row) and tenosynovitis (lower row) for reader 1 (left) and reader 2 (right). The differences (T2set - Gdset) between paired measurements are plotted against the means of the two measurements. The middle line in each graph shows the systematic bias between the two measurement methods. The observation that the line is located around 0 indicates that systematic bias was low. The upper en lower lines show the ± 95% limits of agreement. For tenosynovitis variation increases with higher scores for reader 2.

Agreement for individual joint/tendon scores

Subsequent analyses were performed on joint level with Gdset images as reference. Weighted Kappa's for agreement of synovitis scores in individual joints based on T2set and Gdset were 0.65 (95%CI 0.49-0.81) and 0.71 (95%CI 0.63-0.80) for the two readers, indicating good agreement. For tenosynovitis corresponding values were 0.52 (95%CI 0.36-0.68) and 0.46 (95%CI 0.33-0.60), indicating moderate agreement.

Table 1: 2x2-table, and sensitivity and specificity of assessment of synovitis and tenosynovitis at joint/tendon level and at patient level without contrast injection, with contrast enhanced MRI findings as standard reference.

At joint/tendon level					
Synovitis Reader 1	*Gdset+*	*Gdset-*	*Synovitis Reader 2*	*Gdset+*	*Gdset-*
T2set+	148	56	T2set+	90	29
T2set-	14	58	T2set-	35	122
Sensitivity	91%		Sensitivity	72%	
Specificity	51%		Specificity	81%	
Tenosynovitis Reader 1	*Gdset+*	*Gdset-*	*Tenosynovitis Reader 2*	*Gdset+*	*Gdset-*
T2set+	103	97	T2set+	73	74
T2set-	50	670	T2set-	62	711
Sensitivity	67%		Sensitivity	54%	
Specificity	87%		Specificity	91%	
At patient level					
Synovitis Reader 1	*Gdset+*	*Gdset-*	*Synovitis Reader 2*	*Gdset+*	*Gdset-*
T2set+	78	7	T2set+	45	10
T2set-	3	4	T2set-	13	24
Sensitivity	96%		Sensitivity	78%	
Specificity	36%		Specificity	71%	
Tenosynovitis Reader 1	*Gdset+*	*Gdset-*	*Tenosynovitis Reader 2*	*Gdset+*	*Gdset-*
T2set+	46	24	T2set+	37	13
T2set-	6	16	T2set-	15	27
Sensitivity	89%		Sensitivity	71%	
Specificity	40%		Specificity	68%	

Presence of synovitis and tenosynovitis in individual joints and tendons and in patients with (Gdset) and without (T2set) IV contrast. Synovitis was evaluated in 276 sites (three wrist joints in 92 patients) and tenosynovitis was evaluated in 920 sites (10 wrist compartments in 92 patients) as described in the methods.

Test characteristics on joint/tendon level

The sensitivity to detect synovitis without gadolinium contrast was 91% and 72%, respectively, for the 2 readers and the specificity 51% and 81%. Similarly, for tenosynovitis the sensitivity was 67% and 54% and the specificity 87% and 91% for the two readers (Table 1).

Large discrepancies in scores in individual joints/tendons

Differences ≥ 1 point between T2set and Gdset scores in individual joints or tendons were present in only 1.8% of joints for synovitis and 0.3-0.5% of tendons for tenosynovitis. These cases were reviewed for the cause of this discrepancy. Importantly for synovitis, in all cases areas of high signal on T2 were seen without enhancement on T1Gd images, indicating false-positive results on T2 due to effusion (Fig. 2). For tenosynovitis no clear explanation was found.

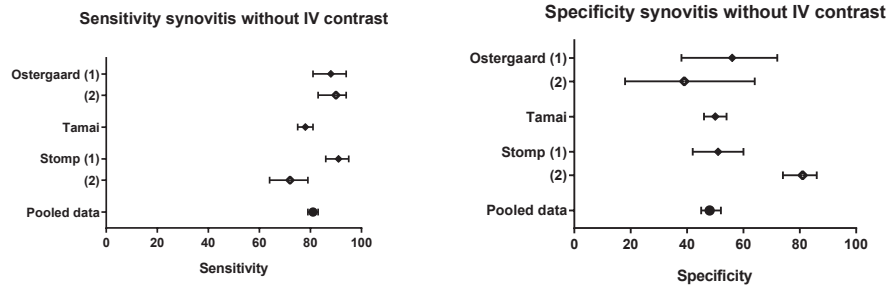


Figure 2: Sensitivity and specificity of evaluation of synovitis without IV contrast in separate studies and in a combined analysis

Plot of sensitivity and specificity estimates of MRI without IV contrast for individual joints and tendons. (A) Sensitivity and (B) specificity. Point estimates of sensitivity and specificity from each study are shown as solid diamonds for the first reader and as open diamonds for the second reader in each study. The solid lines represent 95% CIs.

Literature review and pooled data analysis

Supplementary table 2 lists all studies that were identified and results of each individual study; two studies evaluated synovitis and one other study assessed tenosynovitis with and without contrast.[6–8] The tendency on the findings on joint/tendon level were consistent across studies: low specificity for synovitis; low sensitivity for tenosynovitis. The only exception was assessment of synovitis at 0.2T extremity MRI (as compared to 1.0 or 1.5T for other studies), where sensitivity was low.[7] Figure 2 shows the sensitivity and specificity for synovitis obtained with 1.0/1.5T MRI in different studies. For synovitis, raw data of three studies were pooled on joint level; the overall sensitivity to detect synovitis without

gadolinium was 81% and the overall specificity 50% (Figure 2, Supplementary table 3). For tenosynovitis no pooling could be performed due to differences in the scoring methods used.

DISCUSSION

MRI is sensitive to detect inflammation, but is also time-consuming and costly. We investigated the consequences of eliminating IV gadolinium contrast administration in a cohort of early arthritis patients and subsequently analyzed pooled data from this study and two previously published studies, identified by a literature review. We observed that the sensitivity and specificity were markedly decreased when eliminating the post-IV contrast sequences.

Gadolinium administration adds to the cost and duration of the examination and increases patient discomfort. Furthermore it is contraindicated in patients with severe renal failure due to the risk of nephrogenic systemic fibrosis.[9] For assessment of bone marrow edema and erosions no gadolinium contrast is necessary.[6,7] However, based on our findings and the literature review, IV contrast is necessary for optimal assessment of synovitis and tenosynovitis.

A strength of our study is that we included patients at early disease stage when inflammation is usually limited and MRI may be of additional value in detecting it. Furthermore, we did not limit inclusion to a single diagnosis, which makes our results more widely applicable.

A limitation is that we only assessed wrist joints and not MCP joints. We chose this for time reasons, as we prioritized to acquire axial T2-weighted fat suppressed images in order to have optimal sequences for assessment of synovitis and especially tenosynovitis without contrast injection. Secondly, we only made cross-sectional comparisons, so sensitivity to change, important for clinical trials, could not be compared. However, as cross-sectional data alone documented that non-contrast enhanced sequences cannot replace contrast-enhanced, longitudinal data are less relevant. Finally, our data were obtained in early arthritis patients with relatively low inflammation scores, and may not be generalizable to patients with more advanced disease.

In conclusion, eliminating gadolinium contrast gave a low specificity for synovitis and low sensitivity for tenosynovitis. Consequently, MRI without IV contrast injection cannot be recommended for evaluation of synovitis and tenosynovitis.

REFERENCE LIST

1. Krabben A, Stomp W, Huizinga TW, et al. Concordance between inflammation at physical examination and on MRI in patients with early arthritis. *Ann Rheum Dis* 2013 Dec 12.
2. Østergaard M, Peterfy C, Conaghan P, et al. OMERACT Rheumatoid Arthritis Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. *J Rheumatol* 2003 Jun;30(6):1385–6.
3. Eshed I, Feist E, Althoff CE, et al. Tenosynovitis of the flexor tendons of the hand detected by MRI: an early indicator of rheumatoid arthritis. *Rheumatology (Oxford)* 2009 Aug;48(8):887–91.
4. Haavardsholm EA, Østergaard M, Ejbjerg BJ, et al. Introduction of a novel magnetic resonance imaging tenosynovitis score for rheumatoid arthritis: reliability in a multireader longitudinal study. *Ann Rheum Dis* 2007 Sep;66(9):1216–20.
5. De Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011 Jan;50(1):93–100.
6. Tamai M, Kawakami A, Uetani M, et al. Magnetic resonance imaging (MRI) detection of synovitis and bone lesions of the wrists and finger joints in early-stage rheumatoid arthritis: comparison of the accuracy of plain MRI-based findings and gadolinium-diethylenetriamine pentaacetic acid-enhanced MRI-based findings. *Mod Rheumatol* 2012 Sep;22(5):654–8.
7. Ostergaard M, Conaghan PG, O'Connor P, et al. Reducing invasiveness, duration, and cost of magnetic resonance imaging in rheumatoid arthritis by omitting intravenous contrast injection — Does it change the assessment of inflammatory and destructive joint changes by the OMERACT RAMRIS? *J Rheumatol* 2009 Aug;36(8):1806–10.
8. Tehranzadeh J, Ashikyan O, Anavim A, et al. Enhanced MR imaging of tenosynovitis of hand and wrist in inflammatory arthritis. *Skeletal Radiol* 2006 Nov;35(11):814–22.
9. Thomsen HS, Morcos SK, Almén T, et al. Nephrogenic systemic fibrosis and gadolinium-based contrast media: updated ESUR Contrast Medium Safety Committee guidelines. *Eur Radiol* 2013 Feb;23(2):307–18.

Supplementary table 1: Patient characteristics

	Patients (n=92)	EAC total population (1993-2011, n=2748)	P value
Age, years (mean, SD)	55.8 ±13.5	51.6±17.1	0.02
Female sex, n (%)	49 (53.3)	1640 (59.7)	0.22
Symptom duration in weeks, median (IQR)	13.0 (4.8-29.0)	14.0 (6.0-31.0)	0.67
Swollen joint count (66-SJC), median (IQR)	3.0 (2.0-5.8)	4.0 (2.0-9.0)	0.22
Tender joint count (68-TJC), median (IQR)	6.5 (2.3-10.0)	5.0 (3.0-9.0)	0.21
RF positive, n (%)	28 (30.4)	800 (29.5)	0.85
ACPA positive, n (%)	23 (25.0)	628 (28.0)	0.52
Patient classification at baseline, n (%)			
RA (2010 criteria)	35 (38.0)	1060 (38.6)	0.92[#]
Undifferentiated arthritis	36 (39.1)	827 (30.1)	
Inflammatory osteoarthritis	6 (6.5)	127 (4.6)	
Psoriatic arthritis	7 (7.6)	187 (6.8)	
Other rheumatic diagnoses	8 (8.7)	547 (19.9)	

Except where indicated otherwise, values are number (%) of patients. SD, standard deviation; IQR, interquartile range; 66-SJC, 66 swollen joint count; 68-TJC, 68 tender joint count; RF, Rheumatoid factor; ACPA, anti-citrullinated peptide antibodies. A chi-square test was used for nominal variables and the Student's t test or Mann-Whitney U-test for continuous variables. Student's t test was performed when variables are presented as mean and a Mann-Whitney U-test was performed when variables are presented as median. [#]The frequency of RA versus non-RA was tested.

Supplementary table 2: Overview of literature regarding the need of gadolinium contrast for assessment of synovitis and tenosynovitis

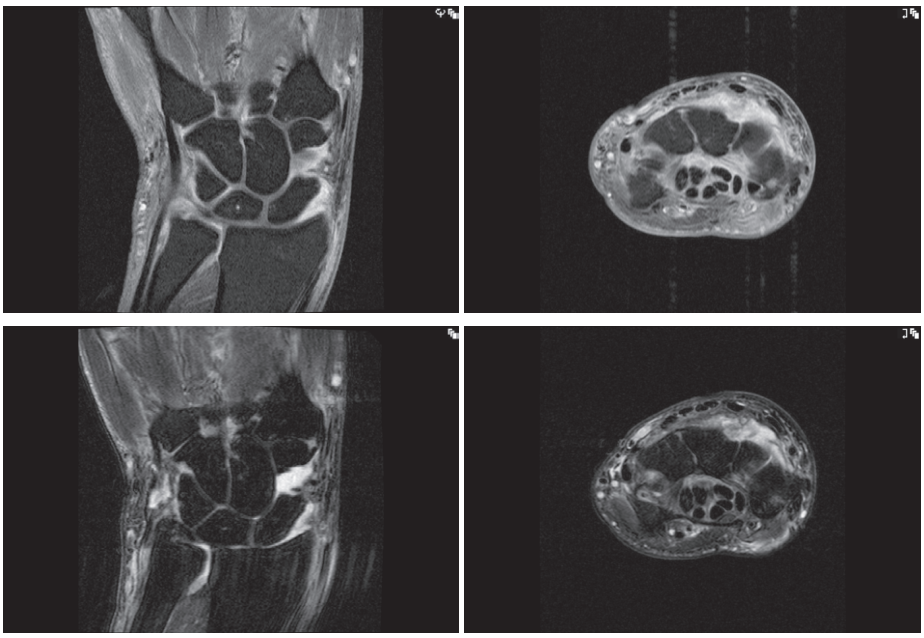
Study	*Patient group*	*MR*	*Joint area*	*Scoring method*	*Disease duration (mean/median)*	*ICC Gd+ vs Gd-*	*Sensitivity / specificity*	*Conclusion*
				Synovitis				
Ostergaard 2009	RA (n=40)	1.0/1.5T	10 wrist/ 30 mcp	RAMRIS	Unspecified	0.63-0.76	86-90% / 31-79%	Omitting IV contrast decreases reliability of synovitis scores
	RA (n=45) healthy (n=9)	0.2T Extremity	Wrist and mcp	RAMRIS	Unspecified	0.61	60% / 96%	
Tamai 2012	Early RA (n=51)	1.5T	Wrist, mcp and pip	RAMRIS	5 months	-	78% / 50%	Synovitis cannot be correctly identified by plain MRI
Current study	Early arthritis (n=92)	1.5T extremity	wrist	RAMRIS	First patient visit	0.75-0.82	77-96% / 36-69%	
				Tenosynovitis				
Tehranzadeh 2006	Inflammatory arthritis (n=30, 72 exams, RA 16, UA 9, PsA 2, CREST 1, SLE 1, paraneoplastic 1)	1.5T	33 wrist/ 39 hand	Self-devised, 0-3	Unspecified	-	40-67% / -	Enhanced MR imaging superior for detection of tenosynovitis.
Current study	Early arthritis (n=92)	1.5T extremity	wrist	RAMRIS	First patient visit	0.57-0.72	71-88% / 40-68%	

All relevant studies found evaluating findings on MRI with compared with MRI without IV contrast administration; current study results also shown. Sensitivity and specificity shown are those reported for individual joints/tendons.

Supplementary table 3: Pooled data from literature for synovitis: 2x2-table, and sensitivity and specificity of assessment of synovitis and tenosynovitis at joint/tendon level without contrast injection, with contrast enhanced MRI findings as standard reference.

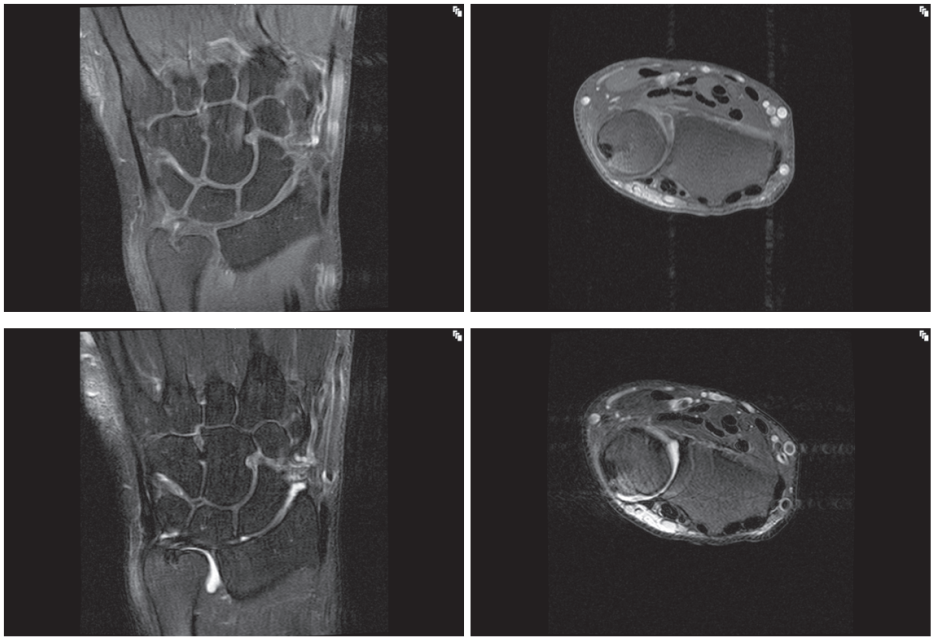
Ostergaard at el.	Gdset+	Gdset-	Total	
T2set+	101	16	117	PPV: 86%
T2set-	13	20	33	NPV: 61%
Total	114	36	150	
	Sensitivity: 89%	Specificity: 56%		
Tamai et al.	Gdset+	Gdset-	Total	
T2set+	613	316	929	PPV: 66%
T2set-	175	312	487	NPV: 64%
Total	788	628	1416	
	Sensitivity: 78%	Specificity: 50%		
Pooled data from Stomp et al, Ostergaard et al and Tamai et al.	Gdset+	Gdset-	Total	
T2set+	862	388	1250	PPV: 69%
T2set-	202	390	592	NPV: 66%
Total	1064	778	1842	
	Sensitivity: 81%	Specificity: 50%		

Number of joints scored positive on T2set and Gdset; data from two other studies and pooled data from the present study as well as studies by Østergaard et al and Tamai et al.(6,7) For studies that reported data on multiple readers, only scores of one reader were used (results were comparable independent of the combination of readers selected). PPV: positive predictive value; NPV: negative predictive value.



Supplementary figure 1a

Example of synovitis and tenosynovitis as visualized by T1-weighted postcontrast and T2-weighted sequences. Upper row: T1-weighted coronal (left) and axial (right) images after gadolinium administration. Bottom row: corresponding T2-weighted coronal (left) and axial (right) images before gadolinium administration. Synovitis of the radioulnar, radiocarpal and intercarpal joints and flexor tenosynovitis is clearly visible on both sequences.



Supplementary figure 1b

Example of large discrepancy in synovitis score between T1-weighted postcontrast and T2-weighted sequences. Upper row: T1-weighted coronal (left) and axial (right) images after gadolinium administration. Bottom row: corresponding T2-weighted coronal (left) and axial (right) images before gadolinium administration. Effusion in the radioulnar and radiocarpal joints results in high signal on T2-weighted images without enhancement on post-gadolinium images.

SUPPLEMENTARY METHODS

MR imaging protocol

MR imaging of wrist was performed within two weeks after inclusion, at the most painful side, or in case of completely symmetric symptoms at the dominant side. The presence of clinical arthritis at physical examination of the wrist was not a prerequisite. MR imaging was performed on a MSK-extreme 1.5T extremity MR imaging system (GE, Wisconsin, USA) using a 100mm coil. The patient was positioned in a chair beside the scanner, with the hand fixed in the coil with cushions.

The following sequences were acquired before contrast injection: T1-weighted FSE sequence in the coronal plane (TR/TE 650/17ms; acquisition matrix 388×88; ETL2); T2-weighted FSE sequence with frequency selective fat saturation in the coronal and axial plane (TR/TE 3000/61.8ms; acquisition matrix, 300x224, ETL7).

After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation in the coronal plane (TR/TE 650/17ms, acquisition matrix 364×224, ETL2), T1-weighted FSE sequence with frequency selective fat saturation in the axial plane (TR/TE 570/7ms; acquisition matrix 320x192; ETL2).

Field-of-view was 100mm. Coronal sequences had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. All axial sequences had 20 slices with a slice thickness of 3mm and a slice gap of 0.3mm. Total imaging time was approximately 25 minutes.

Literature review

For the literature review PubMed was searched with a broad search strategy using the search term ("gadolinium" OR "contrast" OR "enhancement") AND ("synovitis" OR "arthritis" OR "tenosynovitis") AND ("MRI" OR "MR" OR "magnetic resonance"). This yielded 1035 results (November 2013). Abstracts were screened and we selected studies that reported on findings on gadolinium contrast-enhanced images compared to findings on images obtained without gadolinium contrast in MRI of joints of the hand of adult patients with any type of arthritis. For relevant studies (n=3) full-text articles were obtained. Furthermore, references of obtained full-text articles were screened for further relevant studies, which did not yield any additional studies. Of the three studies that were found, two were relevant for synovitis and one for tenosynovitis.

15

Effects of wearing high-heels on the forefoot: an MRI evaluation

W. Stomp, A. Krabben, A. H. M. van der Helm-van Mil, M. Reijnierse

Scandinavian Journal of Rheumatology, 2014;43(1):80-81.

Rheumatoid arthritis frequently involves inflammation of the forefoot. At present Magnetic Resonance Imaging (MRI) is increasingly used, mostly for research purposes, to detect inflammation. Given the sensitivity of MRI, a relevant issue is to discern pathology from normal variations as abnormalities have been observed in healthy persons.[1–4] In particular it is unknown whether regular physical exercise or wearing a particular shoe type affects MRI results. Bone marrow edema has been described in (sometimes asymptomatic) athletes.[5] Since wearing high-heels shifts pressure from the heel to the heads of the metatarsal bones,[6–8] we hypothesized that this might result in acute abnormalities of the forefoot similar to changes secondary to trauma or repetitive stress. We performed a single-blind crossover study to determine this.

Three healthy females (17-18 years) underwent 1.5T MRI examinations of the dominant foot before (8am) and after (5pm) a school-day, with provoked walking distance and visual-analogue-scale pain score. Activities, pain and symptoms were recorded twice every hour. Flat shoes were worn on day 1 and high heels (mean height 9.48cm) on day 2. Subjects wore flat shoes for one week before both examinations. MRI of the forefoot included T_1 weighted and T_2 weighted fat-suppressed scans, both in three orthogonal directions. Evaluation was performed by two readers independently who were blinded to the study day. In case of disagreement a third reader, a musculoskeletal radiologist, took the judgment. The study was approved by the institutional review board and all participants gave their written informed consent.

The subjects walked 6.7, 6.1 and 6.5 km on day 1 and 8.1, 7.0 and 7.1 km on day 2, including 15-minutes of stair climbing. VAS-pain scores remained low (range 0-2) on day 1 but increased to 5-8 at the end of day 2. Pain was predominantly located at the forefoot area. One subject (a fanatic gymnast) had extensive bone marrow edema of the medial sesamoid bone adjacent to the first MTP joint, which was unchanged over all four MRI's. No other abnormalities were seen on all 8am MRIs, and neither on the forefoot MRI at 5pm at day 1. All 5pm MRIs taken on day 2 revealed plantar subcutaneous edema, consistently located at the medial forefoot, extending over 25-34mm proximal to the metatarsophalangeal (MTP) joints (figure 1). Since during high-heel wearing the pressure point is at or distal to the MTP joints, this edema may have been caused by soft tissue strain due to tensile loading.[6] Two subjects also developed subcutaneous edema dorsal to the metatarsal heads, probably as a direct result of entrapment of the forefoot. Importantly, no other changes, such as synovitis, joint effusion, bone marrow edema, enthesitis, (intermetatarsal) bursitis, tenosynovitis or plantar fasciitis, were observed.

Thus, in the present case series we observed that physical exercise of moderate intensity and high heeled exercise does not cause bone marrow edema or deep soft tissue abnormalities. The pain that was experienced when performing physical exercise and wearing high heels was probably related to the presence of subcutaneous edema, plantar typically proximal to the MTP joints. The major limitation of these data is that the number of sub-

jects studied is small. Nonetheless all individuals evaluated showed remarkably consistent results. Altogether, these data therefore imply that wearing high-heels is not associated with short-term structural abnormalities. The long-term effects of high heel-wearing remain unknown. These results are relevant when interpreting forefoot MRI-examinations of rheumatoid or orthopedic patients

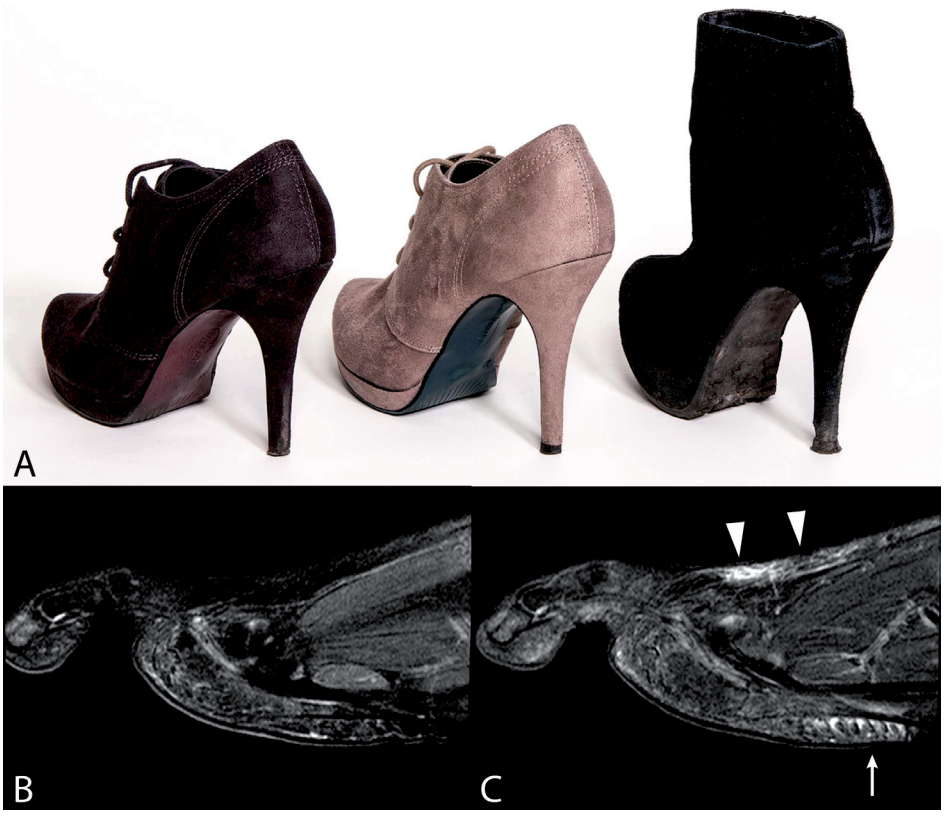


Figure 1: (A) High heels worn by the three subjects. (B,C) Sagittal T2-weighted fat-suppressed image at the level of the second metatarsal bone before (B) and after (C) a day of high heel wearing shows subcutaneous edema at the plantar and dorsal side. Note the proximal location to the MTP joints on the plantar side (arrow).

REFERENCE LIST

1. Ejbjerg B, Narvestad E, Rostrup E, et al. Magnetic resonance imaging of wrist and finger joints in healthy subjects occasionally shows changes resembling erosions and synovitis as seen in rheumatoid arthritis. *Arthritis Rheum* 2004 Apr;50(4):1097–106.
2. Olech E, Crues JV, Yocum DE, et al. Bone marrow edema is the most specific finding for rheumatoid arthritis (RA) on noncontrast magnetic resonance imaging of the hands and wrists: a comparison of patients with RA and healthy controls. *J Rheumatol* 2010;37(2):265–74.
3. Palosaari K, Vuotila J, Soini I, et al. Small bone lesions resembling erosions can frequently be found in bilateral wrist MRI of healthy individuals. *Scand J Rheumatol* 2009;38(6):450–4.
4. Krabben A, Stomp W, van der Heijde DM, et al. MRI of hand and foot joints of patients with anticitrullinated peptide antibody positive arthralgia without clinical arthritis. *Ann Rheum Dis* Published Online First: 19 January 2013.
5. Navas A, Kassarjian A. Bone marrow changes in stress injuries. *Semin Musculoskelet Radiol* 2011; 15(3): 183–97.
6. Ko P-H, Hsiao T-Y, Kang J-H, et al. Relationship between plantar pressure and soft tissue strain under metatarsal heads with different heel heights. *Foot Ankle Int* 2009; 30(11): 1111–6.
7. Snow RE, Williams KR, Holmes GB Jr. The effects of wearing high heeled shoes on pedal pressure in women. *Foot Ankle* 1992; 13(2): 85–92.
8. McBride ID, Wyss UP, Cooke TD, et al. First metatarsophalangeal joint reaction forces during high-heel gait. *Foot Ankle* 1991; 11(5): 282–8.

16

Summary and general conclusion

Rheumatoid arthritis (RA) is an auto-immune disease that is characterized by symmetric polyarthritis of especially the small hand and foot joints. RA is prevalent and has major consequences on physical functioning, which has a big impact on daily life and work ability and also has socio-economic consequences. During the last decades the treatment for RA has dramatically improved, and a lot of new effective drugs have become available. From previous studies we learned that early recognition and treatment will result in a better outcome. This is already applied in daily practice. However, these treatments are not without side-effects and are not for free. Therefore, the question remains how can we discriminate patients/persons that will develop RA from those that will not? What are predictive factors for development of RA? And when a patient has RA what will be his/her prognosis? What are predictive factors for the disease course?

PART I: UNCLASSIFIED ARTHRITIS AND THE RISK ON DEVELOPMENT OF RHEUMATOID ARTHRITIS

In 2010 new classification criteria for RA were established, to classify RA patients earlier in the disease course. Thereafter, several studies focused on the characteristics and outcome of this new group of RA patients. The overall conclusion was that the 2010 criteria allow earlier diagnosis of RA, although also resulting in a bigger group of over diagnosed patients with self-limiting disease.[1-4] However, the group of unclassified arthritis (UA) patients also changed, as a consequence of the new RA group.

In **chapter 2** we evaluated characteristics and outcomes of UA according to the 2010 criteria from the Leiden EAC. We found that 2010-UA patients had milder baseline characteristics than 1987-UA patients. To evaluate the outcome of the UA patients we used different outcomes, because there is no ultimate outcome definition for RA diagnosis. We used three different outcomes that can be seen as surrogates for RA: 1987 criteria fulfillment during the first year, DMARD initiation during the first year and persistent disease on the long term (no remission).

During follow-up, 24% of the 2010-UA patients still fulfilled the 1987 RA criteria compared to 32% of the 1987-UA patients. The 2010-UA patients started less frequent DMARD therapy and reached more frequent sustained DMARD free remission. However, the percentage of UA patients that developed RA during the first year would probably be even higher when looking at the natural course of the disease of these patients, assuming that treatment could have prevented them from developing RA. Although, the majority of these patients were enrolled when aggressive DMARD treatment of UA was uncommon.

In order to determine whether classification according to the 2010 criteria was congruent with risk estimation by the 'Van der Helm prediction rule',[5] both methods were applied to 1987-UA patients. The majority who did not fulfill the 2010 criteria was also in the low

risk group of the prediction rule. However, 30% of 2010 criteria positive patients were predicted to have a low risk on RA and during the course of the disease these patients achieved more frequent DMARD free sustained remission than other 2010 criteria positive patients. Evaluation of congruency of both methods is formally not correct, as the 2010 criteria are meant for classification and the prediction rule is meant to estimate individual patient' probability of fulfilling the 1987 criteria at an early stage. On the other hand, the 2010 criteria will most likely also be used for individual patients in the clinic. Overall can be concluded that UA in the era of the 2010 criteria is less prevalent and milder at presentation and in outcome, but 24% of the 2010-UA patients still fulfill the 1987 criteria within one year, which implies that careful clinical observation of 2010-UA patients is indicated. (Figure 1)

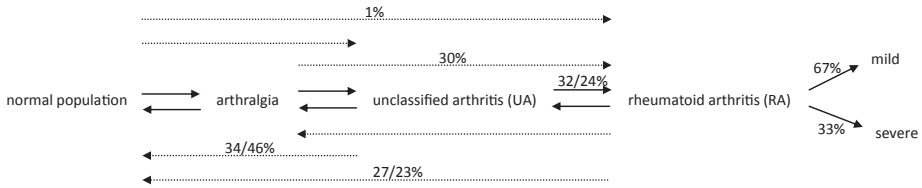


Figure 1 Disease phases of RA, including the percentages of patients evolving from one phase through another. 1% of the normal population develops 1987-RA, adapted from Scott et al (7). 30% of the arthralgia patients (ACPA positive) progress to 1987-RA, adapted from Van de Stadt et al (8). 32% of 1987-UA patients and 24% of 2010-UA patients develop 1987-RA, adapted from chapter 2 of this thesis. 34% of 1987-UA patients and 46% of 2010-UA patients develop remission, adapted from chapter 2 of this thesis. Approximately 23% of 1987-RA patients and 27% of 2010-RA patients develop remission, adapted from v. Nies et al (9). 33% of 1987-RA patients develop (under monotherapy with MTX) RRP and 67% does not, adapted from K. Visser et al (10).

We were quite surprised to find such a high percentage of 2010-UA patients still progressing into RA during the first year. Therefore, in **chapter 3** we also looked at in other cohorts to study the percentage of the 2010-UA patients that develop RA during follow-up. We studied 2010-UA patients from the early arthritis cohorts from Leiden, Birmingham UK and Amsterdam.[1,2,6] This resulted in 24%, 26%, and 12% of the 2010-UA-patients fulfilling the 1987 criteria after one year, respectively. However, some of these patients already fulfilled the 1987 criteria at baseline. In 1987-and-2010-UA patients, 15%, 21%, and 9% respectively developed 1987 RA at one year. Because these early RA patients are missed at baseline, we tried to find prognostic markers for RA development and evaluated the prognostic accuracy of ACPA and the 'Van der Helm prediction rule' in 2010-UA patients. In these 1987-and-2010-UA patients that developed 1987 RA at one year, 0-6% of the patients were ACPA positive and 0-1% had high prediction scores. Consequently, a large majority of the UA patients with an unfavorable outcome was not recognized by

these prognostic tools. Therefore, ACPA and the Leiden prediction rule are not useful in identifying these patients. Because the high percentage of 2010-UA patients still developing RA and these prognostic tools are not useful in this group of patients, we concluded that other predictive markers should be developed for 2010-UA patients.

PART II: GENETIC AND SEROLOGIC FACTORS IN PREDICTING RADIOGRAPHIC PROGRESSION IN RHEUMATOID ARTHRITIS

When a patient is diagnosed with RA the disease course is not yet known, due to a lot of variation in the disease course of RA. Some patients will rapidly progress in a very erosive and disabling disease, while others will develop remission. An objective and commonly used outcome measure of the disease course of RA is the rate of radiographic progression. It is measured most sensitively with hand and foot radiographs that are serially taken over time and scored using a validated quantitative scoring method.

First, in **chapter 4** biomarkers for radiographic progression are summarized in a review. In this review we evaluated the published (and partly non-published) data on genetic, serologic and imaging biomarkers for the severity of joint destruction in RA. In genetics the combination of low prevalence of variations and small effect sizes means that large data sets are necessary. Unfortunately, large data sets on long-term longitudinal radiographic joint destruction are scarce. Furthermore, a relevant question to consider is when a genetic association is true. The first level of evidence is the p-value, which is insufficient to indicate whether a variant can be true. More reliable is replication. In case a variant is statistically significant associated in several independent cohorts, the chance that the observation reflects a chance-finding is importantly reduced. Even more convincing are data that support the finding at a different level, like at mRNA expression or protein level. In the ideal situation the pathway or mechanism via which a genetic risk factor influences the disease is understood.(Figure 2) In this review, genetic variants in 28 genes were evaluated; variants in 10 genes (*CD40, IL2RA, IL4R, IL15, OPG, DKK1, SOST, GRZB, MMP9, SPAG16*) were evidently replicated in independent data sets and for five variants (*IL2RA, DKK1, GRZB, MMP9, SPAG16*) there was also evidence for an association at the functional level. We evaluated several serological biomarkers like; auto-antibodies (RF, ACPA, anti-CarP), markers related to inflammation (ESR, CRP) and proteinases or components of the extracellular matrix of bone or cartilage (MMP3, CTX-I, CTX-II, COMP, TIMP1, PYD, RANKL/OPG, CXCL13). Finally, we evaluated markers that can be visualized by ultrasound or MRI, like; erosions, bone marrow edema, synovitis and tenosynovitis. Several studies showed that bone marrow edema and synovitis detected by MRI are strong predictors for radiographic progression and some showed that inflammation detected with ultrasound also predicted radiographic progression. We also found that serological and imaging markers generally

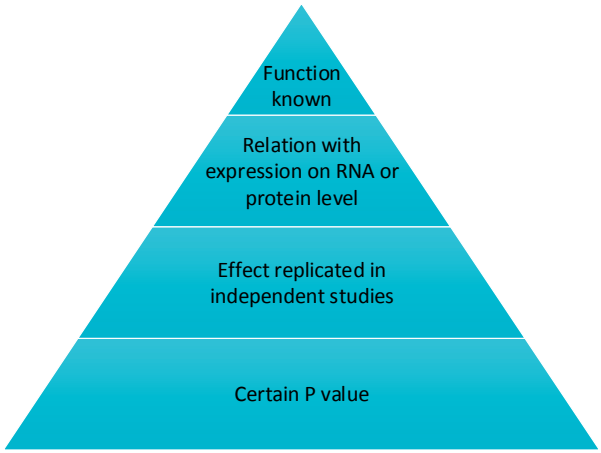


Figure 2 The level of evidence in genetic studies. The higher into the pyramid, the higher the level of evidence.

have larger effect sizes than genetic markers. This might be due to the fact that serological and imaging markers are more closely related to the phenotype. Until now, the majority of these known risk factors have not yet been included in risk models. Future studies will reveal whether adding and combing all these different biomarkers will increase the predictive accuracy of risk models predicting radiographic progression in RA. However, adding a predictive marker should be cost-effective; add additional clinically relevant prediction to the model and should not be too expensive, time consuming or invasive.

In the following chapters four genetic biomarkers and one serologic biomarker for radiographic progression are evaluated. In **chapter 5-7** we investigated four candidate genes for their association with radiographic joint destruction. All four genes were investigated on a candidate gene study approach. This means that all genes were selected on the individual high 'a prior chance' of being associated to radiographic joint destruction. According to literature these genes were more prone to be associated to radiographic progression and therefore these genes were independently tested for an association to radiographic joint destruction. The purpose of these studies was not to find every possible association, but to find true associations of genetic variants. On the one hand this resulted in a low chance of false-positive findings, but on the other hand a high chance of false-negative findings, as this latter aspect was not the aim of these studies. For each gene, these SNPs were selected that tagged the whole gene. In all studies we used a similar strategy. First we tested all tagging SNPs in a first (identification) cohort. As already mentioned earlier (in chapter 4); a significant genetic association in one cohort is not sufficient to indicate whether a genetic association is true. You need at least replication and even more convincing would be data that support the finding at a functional level. Therefore, the second step was selecting the

SNPs that were significantly associated to radiographic joint destruction in phase one and testing these in other (replication) cohorts. When a SNP was also significantly associated to radiographic progression in a replication cohort, the chance of having a false-positive finding will be reduced. Ideally, you would like to confirm that the finding is really true by showing functional data as well.

In **chapter 5** we investigated the association of genetic variants in *IL15* with radiographic progression rate in RA. Several studies showed that IL-15 plays a role in maintaining inflammation and affecting osteoclastogenesis.[11,12] In patients with RA, IL-15 levels are increased in serum, synovium and bone marrow.[13,14] Additionally, serum levels of IL-15 correlated strongly to disease activity.[15] Therefore, we investigated whether genetic variants in *IL15* are associated with the severity of joint destruction in RA. Five SNPs were significantly associated with the rate of radiographic progression in the first phase (Leiden EAC) and were genotyped in three other cohorts (Groningen, Lund (SE) and Sheffield (UK)). Independent replication was not obtained, possibly due to insufficient power in the replication cohorts. However, a meta-analysis of all four data sets resulted in significant results for four SNPs (rs7667746, rs7665842, rs4371699, rs6821171). These SNPs remained significant after correction for multiple testing. This suggests that these four genetic variants in *IL15* are associated with radiographic progression in RA. Further studies should demonstrate whether the presence of these variants results in difference at functional level of IL15.

In **chapter 6** we studied the association between *IL4* and *IL4R* tagging SNPs and the rate of radiographic progression in RA. Previous studies showed that IL-4 has an anti-inflammatory effect, as well as an anti-osteoclastogenic effect.[16,17] The effect of IL-4 is mainly mediated by the IL-4 receptor alpha chain (IL4-Ralpha). Several genetic studies of *IL4* and *IL4R* and joint damage have been performed,[18-21] though none of the factors identified have been replicated. This prompted us to perform this multi cohort candidate gene study. In the first phase (Leiden EAC), none of the *IL4* SNPs and seven of the *IL4R* SNPs were significantly associated with the rate of radiographic progression. These seven SNPs were analyzed in four (Groningen, Lund (SE), Sheffiled (UK), NARAC (US)) or six (Groningen, Lund (SE), Sheffield (UK), NARAC (US), Wichita (US), NDB (US)) other cohorts (depending on the availability of the genotyping of the particular SNPs in the cohorts). In this candidate gene study more cohorts were available in the second phase. Therefore, we could perform an independent replication, which consisted of a meta-analysis of only the additional cohorts in the second phase. In the replication phase, two SNPs in the *IL4R* gene were significantly associated with the rate of radiographic progression. One of these two SNPs remained significant after correcting for multiple testing. This SNP (rs1119132) was recessively associated to joint damage progression, which means that carrying both minor alleles results in more severe joint damage progression and only a minority of RA patients will have this homozygous recessive variant. Nevertheless, we did find an independent replication in some of the replication cohorts as well as in the meta-analysis of the six

replication cohorts. In conclusion, we identified and replicated a genetic variant in *IL4R* predisposing to radiographic progression in RA. Further studies of IL-4R at a functional level are needed to confirm this finding and to increase insight on the role of this variant in the pathogenesis of RA progression.

In **chapter 7** we investigated Granzyme B (*GZMB*) as candidate gene for radiographic progression in RA. GZMB is a serine protease found in the lytic granules of natural killer (NK) cells and cytotoxic T lymphocytes and can induce cell death. Previous studies suggest a role for GZMB in RA joint destruction as well, because the number of GZMB-positive cells is increased in synovium as well as among chondrocytes at the site of a pannus lesion.[22-24] Therefore, we tested whether genetic variants in *GZMB* were associated to radiographic progression. Two SNPs were significantly associated to radiographic progression in the first phase (Leiden EAC). However, these two SNP were highly correlated and therefore only the strongest one (rs8192916) was tested in the second phase. The data available for the second phase were expected to be underpowered for individual replication and therefore this SNP was tested in a meta-analysis of all four datasets (Leiden EAC, Groningen, Lund (SE), Sheffield (UK)), and resulted in a significant association with radiographic progression. This association remained significant after correcting for multiple testing. The minor allele of this SNP was associated with a higher rate of joint destruction. This significant remaining SNP was also tested for its association with RNA expression of the genomic region of *GZMB*. This resulted in a higher expression of GZMB RNA in the presence of the minor allele of this SNP. In conclusion, this suggests that this genetic variant located in *GZMB* is associated with radiographic progression as well as with RNA expression in whole blood in RA.

In **chapter 8** we evaluate the predictive value of serum pyridinoline (PYD) levels for joint destruction. PYD is a major crosslink of collagen in cartilage, bone and synovium. Previous studies showed that PYD levels are increased in patients with RA compared to healthy persons and PYD levels are higher in cases of active or severe disease.[25] Most previous studies were performed on urine levels of PYD. However, no large scale longitudinal studies on serum PYD levels and radiographic progression in RA have been performed. We evaluated the predictive value of serum PYD levels for future joint destruction, both at baseline for long-term prediction and during the disease course for near-term prediction. Evaluating baseline PYD serum levels revealed that these baseline levels were significantly associated with the rate of radiographic progression over seven years and also independent of known risk factors for joint destruction, like; age, sex, treatment, ACPA status, CRP level, BMI and smoking. Furthermore, the PYD levels during follow-up were significantly associated to the rate of radiographic progression the upcoming year and also independent of the known risk factors. Although serum PYD was found to be an independent risk factor, the accuracy (measured by the AUC) of PYD levels on the rate of radiographic progression was moderate. Therefore, increased PYD serum levels, both at baseline and during the disease course,

are associated with a higher rate of radiographic progression during the coming year(s), though the predictive accuracy as a sole predictor was moderate. Therefore, it could be valuable to include serum PYD in combined predictive models to predict radiographic progression in RA patients.

PART III: MRI IN PATIENTS WITH ARTHRALGIA AND EARLY ARTHRITIS

MRI is becoming more and more important in RA research. MRI is highly useful because it is a very sensitive measure, especially for inflammation. An important feature of MRI is the detection of inflammation inside the bone. In this part we investigated the process that is going on in the (partly clinical invisible) early disease stages of RA. Additionally, we investigated whether MRI can distinguish RA patients from other early arthritis patients.

From previous studies we know that ACPA, RF and acute phase reactants are already present years before the first symptoms of RA emerge, which suggests that there is a pre-clinical phase in RA.[26] However, it was still unknown whether local inflammation occurs in the joints in the preclinical phase. A MRI study on knee joints of 13 ACPA positive arthralgia patients showed no subclinical inflammation.[27] However, ACPA positive RA probably does not start in knee joints, leaving the question whether local inflammation is present in the preclinical phase of ACPA positive RA unanswered. In **chapter 9** we studied small joints of 21 ACPA positive arthralgia patients on local subclinical inflammation. The inflammation detected by MRI was compared among; small joints of controls (n=19) (without joint complains), small joints of ACPA positive arthralgia patients and small joints of ACPA positive RA patients (n=22). This study showed that ACPA positive arthralgia patients had more MRI detected inflammation in the PIP/MCP and wrist joints, than controls without joint complains and lower MRI detected inflammation than ACPA positive RA patients. These data suggest that local subclinical inflammation occurs in ACPA positive arthralgia patients. Probably there is already an inflammation process going on in small joints before the joints are clinically inflamed and can be detected by physical examination as swollen joints.

The next question was whether MRI detectable inflammation is congruent to inflammation detected by physical examination in joints of arthritis patients. Possibly there are inflamed joints that are missed by physical examination. In **chapter 10** the association and concordance between inflammation of small joints measured with MRI and physical examination, was determined. We studied 1,790 joints of 179 patients from the Leiden EAC. In these joints; synovitis and tenosynovitis on MRI were independently associated with clinical swelling, in contrast to bone marrow edema (BME). In the majority (~90%) of the swollen MCP joints and swollen wrists any inflammation on MRI was present. In 27% of the non-swollen MCP joints and in 66% of the non-swollen wrists any MRI inflammation was present. Vice versa, of all MCP, wrist and MTP joints with inflammation on MRI 64%,

61% and 77% respectively were not swollen. BME, also in case of severe lesions, occurred frequently in clinically non-swollen joints. Additionally, similar results were observed for joint tenderness. In conclusion, inflammation on MRI is not only present in clinically swollen but also in non-swollen joints. In particular BME occurred in clinically non-inflamed joints. From previous studies we know that BME is an independent predictor of radiographic progression.[28-33] Moreover, although we found a high percentage of joints with subclinical inflammation, we know that MRI is very sensitive and probably in some cases too sensitive. Therefore, we wanted to know what the relevance of subclinical inflammation is for the disease course.

In **chapter 11** we assessed the relevance of this subclinical inflammation with regard to radiographic progression during the first year. The major reasons to choose for radiographic progression as an outcome measure were; a commonly used outcome in RA, its objectiveness and because the score at joint level can be subtracted. Physical functioning or disease activity would be other interesting parameters, but are not useful in analyses at joint level. However, these parameters could be interesting when translating into clinical practice in the future. In this study we first tried to replicate what others already found, to be sure that the data behaves similar and the predefined assumptions also apply on our data set. Indeed, we also found that BME, synovitis and tenosynovitis were associated with radiographic progression, independent of known risk factors. Next, we investigated the relevance of the subclinical inflammation. Of all non-swollen joints, 26% of the joints had subclinical inflammation. Radiographic progression was present in 4% of non-swollen joints with subclinical inflammation compared to 1% of non-swollen joints without subclinical inflammation. Similar observations were done for the inflammation features separately; BME 7% versus 1%, synovitis 5% versus 1% and tenosynovitis 4% versus 1%. Therefore, although radiographic progression was infrequent, joints with subclinical inflammation had an increased risk of radiographic progression within the first year. This demonstrates the relevance of MRI detected subclinical inflammation. Although, it is difficult to translate it into clinical practice, because these analyses were done on joint level and the rheumatologist treats patients and not joints and these joints with subclinical inflammation were distributed among 91% of the patients. Therefore, this study mainly increases the comprehension of the connection between inflammation and structural damage early in the disease.

The EULAR taskforce recently suggested that MRI can improve the certainty of the diagnosis RA.[34] Since this recommendation may reflect a tendency to use MRI in daily practice, thorough studies on the value of MRI are required. Thus far no large studies have evaluated the accuracy of MRI to differentiate early RA from other early arthritis patients. In **chapter 12** we performed a large cross-sectional study to determine if patients that are clinically classified with RA differ in MRI features compared to patients with other diagnoses.

We observed that among all patients presenting with early arthritis, RA patients had significant higher scores for synovitis, tenosynovitis and BME than non-RA patients. Although for all MRI inflammation features the negative predictive value for RA was good (>0.80), the positive predictive value for RA was low (<0.50). For all MRI features the AUCs were <0.70. Additionally we compared ACPA positive and negative UA and RA patients and found that ACPA positive patients had more BME than ACPA negative patients. From previous studies we know that BME is a strong independent predictor for progression of joint destruction and therefore this is in line with ACPA positive RA being a more severe disease.[35] Furthermore, we compared the patients fulfilling the 2010 and 1987 criteria for RA, by dividing them in four groups, either fulfilling both sets, only one set or none. We observed that patients fulfilling the 2010 criteria but not the 1987 criteria had less synovitis. This suggests that patients that only fulfill the 2010 criteria have a milder disease, which is in line with the results of previous studies.

In conclusion, although RA patients had higher scores of MRI inflammation and ACPA positive patients had more BME, the severity of MRI inflammation does not accurately differentiate RA patients from other early arthritis patients. However, particularly interesting is whether MRI can have additional value in the group of patients that are classified as UA and will develop into RA. Therefore, large longitudinal studies are required to determine whether MRI is valuable for classification of patients that are clinically undefined.

PART IV: MRI SCAN PROTOCOL REVISITED

In this part the scan protocol for MRI is revisited. The OMERACT MRI in RA working group developed and validated a semi-quantitative scoring method system (RAMRIS).[36-38] The scoring system recommends having at least the following sequences: imaging in two planes with T1-weighted images before and after intravenous gadolinium contrast and a T2-weighted fat saturated sequence. This set of sequences is very time consuming and therefore costly and not patient friendly. Therefore, ideally less sequences and no contrast administration is necessary.

On MRI BME can be observed due to the focally increased water content in the bone marrow, partly or entirely replacing normal bone marrow fat. Therefore, BME signal intensity is low on T1 weighted sequences and high on T2 weighted fat saturated sequences. Additionally, BME also enhances with intravenous gadolinium on T1 weighted fat saturated sequences after gadolinium contrast administration and are very similar to T2 weighted fat saturated sequences with regard to BME presentation.

In **chapter 13** we evaluated whether T1 weighted post-gadolinium images (T1Gd) can replace T2 weighted images (T2) for evaluating BME. We observed that the total BME scores did not differ between the two sequences (T1Gd and T2). Intra-reader ICC's be-

tween scores based on T2 and T1Gd images were all excellent (0.80-0.99). The sensitivity and specificity of BME on T1Gd compared to T2 was high. Inter-reader ICC's were excellent regardless of the image set used (all>0.83).

In conclusion, on T1Gd images equal scores for bone marrow edema were scored compared to the standard T2 images. Therefore, for RAMRIS scoring, a short protocol of T1 and T1 fat saturated post gadolinium sequences might be sufficient. This results in a 20% reduction of imaging time. Additionally, T2 weighted sequences are more prone, than T1 weighted sequences, to artifacts due to magnetic field inhomogeneity and movement. Especially when imaging multiple joint areas in one session, shortening of the imaging time in combination with more robust sequences decreases the chance of unsuccessful MR examinations.

According to the RAMRIS protocol and the additional protocol for tenosynovitis scoring by Haavardsholm, intravenous contrast is administrated to assess synovitis and tenosynovitis on the T1 weighted post contrast images. However, synovitis and tenosynovitis normally exhibit high signal intensity both on T2 weighted and T1 weighted post contrast images. In **chapter 14** we studied whether intravenous contrast administration can be eliminated from the scanning protocol, decreasing imaging time, cost and invasiveness.

At individual joint/tendon level, compared to contrast-enhanced images, sensitivities to detect synovitis without contrast were 72% and 91% for both readers and the specificities 52% and 81%. Similarly, for tenosynovitis the sensitivities were 54 and 67% and specificities 88% and 91%. A review of literature showed overall high sensitivity and low specificity to evaluate synovitis and low sensitivity and high specificity to evaluate tenosynovitis without contrast-enhancement. An explanation for the high sensitivity and low specificity for scoring synovitis on T2 weighted images, might be due to the fact that on T2 weighted images hyperplastic inflamed synovium could not be adequately differentiated from fluid in the joints. In conclusion, eliminating intravenous contrast administration decreases specificity for synovitis and sensitivity for tensosynovitis, indicating that intravenous contrast administration remains essential for an optimal assessment.

An additional remark on comparing MRI scoring on different sequences is that BME, synovitis and tenosynovitis scoring on MRI according to the RAMRIS method does not imply there will always be inflammation present that can be seen as pathology. MRI is known to be very sensitive and therefore low grade scoring of these features could also be physiological normal variations. Therefore, these results of scoring on other sequences, then were recommended by the RAMRIS method, do not automatically imply that scoring should result in worse results. More important is the clinical consequence of the score and

another important measure is sensitivity to change, which could be an important measure in clinical trials.

In chapter 15 we investigated the possible influence of a normal daily intervention on the images made with MRI. As wearing high heels shifts pressure from the heel to the heads of the metatarsal bones, we hypothesized that this might result in acute abnormalities of the forefoot similar to changes secondary to trauma or repetitive stress. We performed a single-blind crossover study to determine this. Flat shoes were worn on the first day and high heels on the second day and MRI was made twice on both days; at the beginning of the day and at the end of the day. No BME or deep soft tissue abnormalities were caused by wearing high heels for a day. The only changed abnormality was revealed on all MRI scans taken at the end of day two; plantar subcutaneous edema, consistently located at the medial forefoot extending proximal to the MTP joints. These data imply that wearing high heels is not associated with short-term structural abnormalities. The long-term effects of wearing high heels remain unknown. These results are relevant when interpreting forefoot MRI examinations of rheumatoid or orthopedic patients.

GENERAL CONCLUSION

In this thesis we tried to answer the following questions:

1) How can we discriminate patients/persons that will develop RA from those that will not? What are predictive factors for development of RA?
2) What will be the prognosis of a RA patient? What are predictive factors for the disease course of RA?

1) How can we discriminate patients/persons that will develop RA from those that will not? What are predictive factors for development of RA?

In chapter 12, we found that among early arthritis patients RA patients had higher MRI inflammation scores. However, the accuracy to differentiate RA patients from other early arthritis patients was low. Nevertheless, in clinical practice patients will first be classified according to clinical and serological findings, which will result in a remaining group only consisting of UA patients who can possibly be differentiated by MRI findings.

In chapter 2 and 3 we looked into differentiating of UA patients. We found that even after applying the new, more sensitive, 2010 criteria set for RA to early arthritis patients, 24% still developed RA during the first year. This showed the lack of the 2010 criteria in classifying all RA patients at baseline. We tried to find prediction factors for 2010-UA patients that developed RA. However, ACPA positivity and the 'van der Helm-prediction model' could not differentiate these patients.

In chapter 9, we studied patients in an even earlier phase then UA, we studied ACPA positive arthralgia patients. We found that in this early phase of the disease local subclinical inflammation was already present on MRI. This confirms the existence of a pre-clinical phase of RA and the importance of MRI by detecting subclinical inflammation. Therefore, MRI might be of help in predicting RA development in this phase of the disease.

2) What will be the prognosis of a RA patient? What are predictive factors for the disease course of RA?

In chapter 10 and 11 we also found subclinical inflammation in the clinical non-swollen joints in early arthritis patients. To investigate the relevance of this subclinical inflammation in these clinically non-swollen joints, we looked for radiographic progression after one year. We found that joints with subclinical inflammation had an increased risk of radiographic progression. This suggests that MRI detected subclinical inflammation is relevant and can predict for a more severe disease course.

In chapter 5 to 7 we found four *IL15*, one *IL4R* and one *GZMB* SNPs that were associated to a more severe disease course. Additionally, in chapter 8 we found pyridinoline, a serological marker, associated to a more severe disease course.

Furthermore, in chapter 13 to 15 we investigated the MRI scanning protocol, to scan more efficient and accurate. We found that BME could also be scored on the T1 weighted sequence after contrast administration and therefore the scanning protocol can be reduced by eliminating the T2 weighted sequences. We also found that the sequences after contrast administration were essential for optimal assessment of synovitis and tenosynovitis. Furthermore, we showed that wearing high heels is not associated with structural abnormalities on the short time.

FUTURE PERSPECTIVES/CHALLENGES

During the last decades treatment of RA has become very effective, which has resulted in a less severe outcome of RA, with very minor radiographic progression. Furthermore, as we know from previous research early intervention might alter the natural course of RA. This is why the future of RA should be focused on **identifying patients earlier in the disease**, and treating or possibly first tight monitoring of very early RA. This is why research should be focused on identifying predictors for RA development. This is done frequently in patients classified as UA, but the opportunities are in the even earlier phases of the disease. We know from previous studies that anti-bodies and acute phase reactants are already present years before the first symptoms, which suggest there is a pre-clinical phase in RA. However, predictors for RA development in a very early phase can be very specific, but

the challenge is to remain sensitive. For example, arthralgia patients and especially ACPA and/or RF positive arthralgia patients have an elevated risk of developing RA, but when a patient also has arthritis (UA patient) the chance of developing RA will be even higher. The further away from the full blown RA phenotype, the lower the risk of progressing into RA and the more difficult it is to differentiate.

Another important focus point in RA research should be **personalized medicine**. The one RA patient does not exist. Firstly, future research should therefore be focused on predicting the disease course of the individual patient. Secondly, research should be focused on predicting treatment responsiveness (and adverse reactions) in the individual patients. These two should then be combined to make a personalized treatment decision in each RA patient. I expect that in the near future there will probably be sophisticated tools to calculate which treatment step should be taken in each patient, based on the prediction of his/her disease course and the prediction of his /her responsiveness to each treatment.

Another important focus in RA research is already **MRI**, but might become even more important. MRI is a very sensitive tool to measure (sub)clinical inflammation. Furthermore, MRI can measure BME, which is a very strong predictor of progressive disease. Therefore, I expect that MRI could become an important tool for therapeutic decision making and predicting prognosis. In clinical trials MRI will be even more important to measure the impact of disease-suppressing therapy on the course of synovitis and bone marrow edema, because some studies already showed sub-clinical inflammation in patients in clinical remission.[39-43]

Future research is necessary to face these challenges and this might eventually lead to the no longer existence of RA.

REFERENCE LIST

1. Britsemmer K, Ursum J, Gerritsen M, et al. Validation of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: slight improvement over the 1987 ACR criteria. *Ann Rheum Dis* 2011 Aug;70(8):1468-70.
2. Cader MZ, Filer A, Hazlehurst J, et al. Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort. *Ann Rheum Dis* 2011 Jun;70(6):949-55.
3. de Hair MJ, Lehmann KA, van de Sande MG, et al. The clinical picture of rheumatoid arthritis according to the 2010 American College of Rheumatology/European League Against Rheumatism criteria: is this still the same disease? *Arthritis Rheum* 2012 Feb;64(2):389-93.
4. Radner H, Neogi T, Smolen JS, et al. Performance of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2014 Jan;73(1):114-23.
5. van der Helm-van Mil AH, le Cessie S, van Dongen H, et al. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum* 2007 Feb;56(2):433-40.
6. de Rooy DP, van der Linden MP, Knevel R, et al. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? *Rheumatology (Oxford)* 2011 Jan;50(1):93-100.
7. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet* 2010 Sep 25;376(9746):1094-108.
8. van de Stadt LA, van der Horst AR, de Koning MH, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. *Ann Rheum Dis* 2011 Jan;70(1):128-33.
9. Burgers LE, van Nies JA, Ho LY, et al. Long-term outcome of rheumatoid arthritis defined according to the 2010-classification criteria. *Ann Rheum Dis* 2014 Feb 1;73(2):428-32.
10. Visser K, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, et al. A matrix risk model for the prediction of rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the BeSt study. *Ann Rheum Dis* 2010 Jul;69(7):1333-7.
11. Djaafar S, Pierroz DD, Chicheportiche R, et al. Inhibition of T cell-dependent and RANKL-dependent osteoclastogenic processes associated with high levels of bone mass in interleukin-15 receptor-deficient mice. *Arthritis Rheum* 2010 Nov;62(11):3300-10.
12. McInnes IB, Liew FY. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. *Immunol Today* 1998 Feb;19(2):75-9.
13. Kuca-Warnawin E, Burakowski T, Kurowska W, et al. Elevated number of recently activated T cells in bone marrow of patients with rheumatoid arthritis: a role for interleukin 15? *Ann Rheum Dis* 2011 Jan;70(1):227-33.
14. Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. *J Exp Med* 1995 Mar 1;181(3):1255-9.
15. Petrovic-Rackov L, Pejnovic N. Clinical significance of IL-18, IL-15, IL-12 and TNF-alpha measurement in rheumatoid arthritis. *Clin Rheumatol* 2006 Jul;25(4):448-52.
16. Miossec P, Naviliat M, Dupuy dA, et al. Low levels of interleukin-4 and high levels of transforming growth factor beta in rheumatoid synovitis. *Arthritis Rheum* 1990 Aug;33(8):1180-7.
17. Tunyogi-Csapo M, Kis-Toth K, Radacs M, et al. Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: fibroblast-mediated pathologic bone resorption. *Arthritis Rheum* 2008 Aug;58(8):2397-408.
18. Burgos PI, Causey ZL, Tamhane A, et al. Association of IL4R single-nucleotide polymorphisms with rheumatoid nodules in African Americans with rheumatoid arthritis. *Arthritis Res Ther* 2010;12(3):R75.

19. Genevay S, Di Giovine FS, Perneger TV, et al. Association of interleukin-4 and interleukin-1B gene variants with Larsen score progression in rheumatoid arthritis. *Arthritis Rheum* 2002 Jun 15;47(3):303-9.
20. Marinou I, Till SH, Moore DJ, et al. Lack of association or interactions between the IL-4, IL-4Ralpha and IL-13 genes, and rheumatoid arthritis. *Arthritis Res Ther* 2008;10(4):R80.
21. Prots I, Skapenko A, Wendler J, et al. Association of the IL4R single-nucleotide polymorphism I50V with rapidly erosive rheumatoid arthritis. *Arthritis Rheum* 2006 May;54(5):1491-500.
22. Ronday HK, van der Laan WH, Tak PP, et al. Human granzyme B mediates cartilage proteoglycan degradation and is expressed at the invasive front of the synovium in rheumatoid arthritis. *Rheumatology (Oxford)* 2001 Jan;40(1):55-61.
23. Saito S, Murakoshi K, Kotake S, et al. Granzyme B induces apoptosis of chondrocytes with natural killer cell-like cytotoxicity in rheumatoid arthritis. *J Rheumatol* 2008 Oct;35(10):1932-43.
24. Trapani JA, Sutton VR. Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Curr Opin Immunol* 2003 Oct;15(5):533-43.
25. Astbury C, Bird HA, McLaren AM, et al. Urinary excretion of pyridinium crosslinks of collagen correlated with joint damage in arthritis. *Br J Rheumatol* 1994 Jan;33(1):11-5.
26. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004 Feb;50(2):380-6.
27. van de Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. *Ann Rheum Dis* 2011 May;70(5):772-7.
28. Boyesen P, Haavardsholm EA, Ostergaard M, et al. MRI in early rheumatoid arthritis: synovitis and bone marrow oedema are independent predictors of subsequent radiographic progression. *Ann Rheum Dis* 2011 Mar;70(3):428-33.
29. Haavardsholm EA, Boyesen P, Ostergaard M, et al. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. *Ann Rheum Dis* 2008 Jun;67(6):794-800.
30. Hetland ML, Ejbjerg B, Horslev-Petersen K, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). *Ann Rheum Dis* 2009 Mar;68(3):384-90.
31. Hetland ML, Stengaard-Pedersen K, Junker P, et al. Radiographic progression and remission rates in early rheumatoid arthritis - MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial. *Ann Rheum Dis* 2010 Oct;69(10):1789-95.
32. McQueen FM, Stewart N, Crabbe J, et al. Magnetic resonance imaging of the wrist in early rheumatoid arthritis reveals progression of erosions despite clinical improvement. *Ann Rheum Dis* 1999 Mar;58(3):156-63.
33. McQueen FM, Benton N, Perry D, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. *Arthritis Rheum* 2003 Jul;48(7):1814-27.
34. Colebatch AN, Edwards CJ, Ostergaard M, et al. EULAR recommendations for the use of imaging of the joints in the clinical management of rheumatoid arthritis. *Ann Rheum Dis* 2013 Jun;72(6):804-14.
35. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;7(5):R949-R958.
36. Ostergaard M, Edmonds J, McQueen F, et al. An introduction to the EULAR-OMERACT rheumatoid arthritis MRI reference image atlas. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i3-i7.
37. Ejbjerg B, McQueen F, Lassere M, et al. The EULAR-OMERACT rheumatoid arthritis MRI reference image atlas: the wrist joint. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i23-i47.

38. Conaghan P, Bird P, Ejbjerg B, et al. The EULAR-OMERACT rheumatoid arthritis MRI reference image atlas: the metacarpophalangeal joints. *Ann Rheum Dis* 2005 Feb;64 Suppl 1:i11-i21.
39. Brown AK, Quinn MA, Karim Z, et al. Presence of significant synovitis in rheumatoid arthritis patients with disease-modifying antirheumatic drug-induced clinical remission: evidence from an imaging study may explain structural progression. *Arthritis Rheum* 2006 Dec;54(12):3761-73.
40. Brown AK, Conaghan PG, Karim Z, et al. An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* 2008 Oct;58(10):2958-67.
41. Gandjbakhch F, Foltz V, Mallet A, et al. Bone marrow oedema predicts structural progression in a 1-year follow-up of 85 patients with RA in remission or with low disease activity with low-field MRI. *Ann Rheum Dis* 2011 Dec;70(12):2159-62.
42. Gandjbakhch F, Conaghan PG, Ejbjerg B, et al. Synovitis and osteitis are very frequent in rheumatoid arthritis clinical remission: results from an MRI study of 294 patients in clinical remission or low disease activity state. *J Rheumatol* 2011 Sep;38(9):2039-44.
43. Gandjbakhch F, Haavardsholm EA, Conaghan PG, et al. Determining a magnetic resonance imaging inflammatory activity acceptable state without subsequent radiographic progression in rheumatoid arthritis: results from a followup MRI study of 254 patients in clinical remission or low disease activity. *J Rheumatol* 2014 Feb;41(2):398-406.

Nederlandse samenvatting

Reumatoïde artritis (RA) is een auto-immuun ziekte die wordt gekenmerkt door symmetrische polyartritis van vooral de kleine hand- en voet gewrichten. RA komt in 0,5-1% van de bevolking voor en kan grote consequenties voor het fysieke functioneren van de patiënt en zijn/haar dagelijkse leven hebben. Daarnaast kunnen de sociaal-economische consequenties voor de samenleving van significant belang zijn. De afgelopen jaren is de behandeling van RA drastisch verbeterd en is er veel nieuwe en effectieve medicatie beschikbaar gekomen. Verder hebben we uit eerdere studies geleerd dat vroege herkenning en behandeling resulteert in een betere uitkomst, dat tegenwoordig al wordt toegepast in de klinische praktijk. Deze behandelingen zijn echter niet zonder bijwerkingen en bepaald niet goedkoop. Daarom zijn er belangrijke vragen die beantwoord moeten worden, zoals: Hoe kunnen we patiënten/personen die RA gaan ontwikkelen onderscheiden van de patiënten/personen die geen RA ontwikkelen? Wat zijn voorspellende factoren voor het ontwikkelen van RA? Wat is de prognose van een patiënt met RA? Wat zijn voorspellende factoren voor het verloop van de ziekte?

DEEL I: ONGECLASSIFICEERDE ARTRITIS EN HET RISICO VOOR HET ONTWIKKELEN VAN RHEUMATOÏDE ARTRITIS

In 2010 is er nieuwe set van classificatie criteria voor RA tot stand gekomen, om RA patiënten in een eerder stadium van de ziekte te kunnen classificeren. Daarna kwamen er verschillende studies die zich richtten op het onderzoeken van de kenmerken en uitkomsten van deze nieuwe groep RA patiënten. De gemeenschappelijke conclusie was dat de 2010-criteria patiënten in een eerder stadium van de ziekte als RA kan classificeren, maar met als gevolg een grotere groep over-geclassificeerde patiënten met 'self limiting disease'. Als gevolg van de veranderingen in de nieuwe groep RA patiënten, verandert de groep ongeclassificeerde artritis (UA) patiënten ook.

In **hoofdstuk 2** hebben we de kenmerken en uitkomsten van UA volgens de 2010-criteria bekeken in de Leiden EAC. We vonden dat 2010-UA patiënten mildere baseline kenmerken hadden dan 1987-UA patiënten. Om de uitkomsten van de UA patiënten te evalueren, gebruikten we verschillende uitkomstmaten. Dit deden we omdat er geen ultieme uitkomst definitie voor RA bestaat. We gebruikten drie verschillende uitkomsten die kunnen worden gezien als surrogaten voor RA: Zij die voldoen aan de 1987 criteria gedurende het eerste jaar, zij die een DMARD starten gedurende het eerste jaar en zij die een blijvende ziekte

hebben op de langere termijn (geen remissie). Gedurende de follow-up, voldeden 24% van de 2010-UA patiënten alsnog aan de 1987 RA criteria, vergeleken met 32% van de 1987-UA patiënten. De 2010-UA patiënten startten minder vaak een DMARD behandeling en bereikten vaker een blijvende DMARD vrije remissie. Maar het percentage UA patiënten dat RA ontwikkelt gedurende het eerste jaar zou waarschijnlijk hoger zijn als we naar het natuurlijk verloop van de ziekte zouden kijken, aangenomen dat de behandeling voorkomt dat zij RA ontwikkelen. Ook al is de meerderheid van deze patiënten geïncludeerd in de tijd dat een agressieve DMARD behandeling nog niet gebruikelijk was.

Vervolgens keken we of de classificatie volgens de 2010 criteria congruent was aan de risicoschatting door het 'Van der Helm voorspel model'. Beide methoden werden toegepast op 1987-UA patiënten. Het merendeel van de 1987-UA patiënten dat niet voldeed aan de 2010 criteria viel in de laagrisicogroep van het voorspel model. Terwijl maar 30% van de 2010 criteria positieve patiënten in de laagrisicogroep. Deze groep patiënten bereikte vaker DMARD vrije remissie dan de andere 2010 criteria positieve patiënten. Wel moet worden gezegd dat evaluatie van congruentie tussen beiden methoden formeel niet correct is, omdat de 2010 criteria set bedoeld is voor classificatie en het voorspel model bedoeld is voor individuele voorspelling. Toch zullen de 2010 criteria ook worden toegepast op individuele patiënten in de kliniek. In het algemeen kan worden geconcludeerd dat UA in het perspectief van de 2010 criteria, minder vaak voorkomt en milder is in voorkomen en in uitkomst, maar dat nog steeds 24% van de 2010-UA patiënten binnen 1 jaar aan de 1987 criteria voldoet. Dit betekent dat 2010-UA patiënten klinisch goed vervolgd moeten worden.

We waren nogal verrast over het feit dat een zo hoog percentage 2010-UA patiënten nog RA ontwikkelt gedurende het eerste jaar. Daarom hebben wij in **hoofdstuk 3** ook in andere cohorten gekeken naar het percentage 2010-UA patiënten dat RA ontwikkelt gedurende follow-up. We keken naar 2010-UA patiënten uit vroege artritis cohorten van Leiden, Birmingham (UK) en Amsterdam. Daaruit bleek dat respectievelijk 24%, 26% en 12% van de 2010-UA patiënten aan de 1987 criteria voldeed na 1 jaar. Een deel van deze patiënten voldeed echter al aan deze 1987 criteria op baseline. Van de patiënten die zowel 1987- en 2010-UA waren, voldeed na 1 jaar nog respectievelijk 15%, 21% en 9%, aan de 1987 criteria. Omdat deze vroege RA patiënten worden gemist op baseline, probeerden wij voorspellende factoren voor RA ontwikkeling te vinden. In deze context evalueerden wij de voorspellende accuraatheid van anti-cyclisch gecitrullineerde proteïnen (ACPA) en 'het Van der Helm voorspel model' in 2010-UA patiënten. Van de 1987-en 2010-UA patiënten die 1987 RA ontwikkelden gedurende het eerste jaar, was 0-6% ACPA positief en 0-1% had een hoge score volgens het voorspel model. Hieruit kan geconcludeerd worden dat het grootste deel van de UA patiënten die een slechte uitkomst hebben, niet worden herkend door deze voorspellende factoren. Daarom hebben ACPA en het voorspel model geen toegevoegde waarde in het identificeren van deze patiënten. Omdat een dusdanig

hoog percentage 2010-UA patiënten RA ontwikkelt en deze voorspellende factoren geen toegevoegde waarde hebben bij deze patiënten, concluderen wij dat er andere voorspellende factoren voor RA ontwikkeld moeten worden.

DEEL II: GENETISCHE EN SEROLOGISCHE FACTOREN VOOR HET VOORSPELLEN VAN TOENAME VAN RADIOLOGISCHE GEWRICHTSSCHADE IN RHEUMATOÏDE ARTRITIS

Als er bij een patiënt RA wordt gediagnosticeerd is het ziekte verloop nog onbekend, want RA kent een grote variatie in ziekte verloop. Sommige patiënten hebben een zeer progressief ziekteverloop, naar een erosieve en immobiliserende ziekte, terwijl anderen remissie bereiken. Een objectieve en vaak gebruikte methode om de ziekte toename in RA te meten is doormiddel van de toename van radiologische gewrichtsschade. De meest accurate methode om dit te meten is met röntgenfoto's van zowel handen als voeten die op meerdere tijdstippen zijn gemaakt en gescoord volgens een gevalideerde en kwantitatieve scoring methode.

Allereerst worden in **hoofdstuk 4** biomarkers voor toename van radiologische gewrichtsschade besproken in een review. Hierin bespreken we gepubliceerde (en deels ongepubliceerde) data van genetische, serologische en beeldvormende biomarkers voor de ernst van gewrichtsschade in RA. In de genetica zorgt de combinatie van lage prevalentie van variaties en een klein effect ervoor dat grote datasets nodig zijn. Helaas zijn grote datasets met longitudinale radiologische gewrichtsschade data op lange termijn zeldzaam. Verder is een interessante vraag: wanneer is een genetische associatie waar. Het eerste mogelijke bewijs is de p-waarde, die alleen niet voldoende aantoont dat een variant waar is. Betrouwbaarder is replicatie. Als een variant statistische significant geassocieerd is in meerdere onafhankelijke cohorten, is de kans dat de bevinding op toeval berust drastisch verkleind. Nog overtuigender is data die de bevinding op een ander niveau ondersteunt, zoals op mRNA of eiwit niveau. In de meest ideale situatie wordt ook het 'pathway' of het mechanisme begrepen via welke de genetische risicofactor de ziekte beïnvloedt. In dit review worden genetische varianten in 28 genen geëvalueerd; varianten in 10 genen (*CD40, IL2RA, IL4R, IL15, OPG, DKK1, SOST, GRZB, MMP9, SPAG16*) werden gerepliceerd in onafhankelijke data sets en voor vijf varianten (*IL2RA, DKK1, GRZB, MMP9, SPAG16*) werd ook bewijs gevonden voor een associatie op functioneel niveau. We evalueerden zeven serologische varianten, zoals; auto-antilichamen (RF, ACPA, anti-CarP), markers gerelateerd aan inflammatie (BSE, CRP) en proteasen of componenten van de extracellulaire matrix van bot en kraakbeen (MMP3, CTX-I, CTX-II, COMP, TIMP1, PYD, RANKL/OPG, CXCL13). Tenslotte hebben we ook gekeken naar markers die zichtbaar gemaakt kunnen worden door echo of MRI, zoals; erosies, beenmerg oedeem, synovitis en tenosynovitis.

Meerdere studies hebben laten zien dat beenmerg oedeem en synovitis zichtbaar op MRI sterke voorspellers zijn voor toename van radiologische gewrichtsschade en anderen laten zien dat inflammatie op echo hier ook voor voorspelt. Tijdens het evalueren van al deze biomarkers zagen wij dat serologische en beeldvormende markers grotere effecten hadden dan genetische markers. Dit kan komen doordat serologische en beeldvormende biomarkers dichter bij het fenotype staan. Tot op heden wordt het merendeel van deze bekende risicofactoren niet toegevoegd aan risicomodellen. Toekomstige studies zullen moeten aantonen of het toevoegen en combineren van deze verschillende biomarkers de voorspellende accuraatheid van risicomodellen die de toename van radiologische gewrichtsschade in RA voorspellen, zal verbeteren. Maar voordat een voorspellende marker kan worden toegevoegd, moet de marker ook kosteneffectief zijn; de marker moet klinisch relevante voorspelling toevoegen, maar ook niet te duur zijn, te veel tijd in beslag nemen of te invasief zijn.

In de volgende vier hoofstukken worden vier genetische en één serologische biomarker voor toename van radiologische gewrichtsschade geëvalueerd. In **hoofstuk 5-7** onderzochten we vier kandidaat genen op hun associatie met radiologische gewrichtsschade. Alle vier de genen werden onderzocht volgens een kandidaat gen studiebenadering. Dit houdt in dat alle genen zijn geselecteerd op basis van de individuele hoge vooral kans op het hebben van een associatie met radiologisch gewrichtsschade. Volgens de literatuur hebben deze genen een verhoogde kans op een associatie met radiologische gewrichtsschade. Het doel van deze studies was niet het vinden van elke mogelijke associatie, maar het vinden van ware associaties van genetische varianten. Aan de ene kant resulteerde dit in een kleine kans op het vinden van fout-positieve associaties, maar aan de andere kant een grote kans op het vinden van fout-negatieve associaties. Hoewel dit laatste ook niet het doel van de studies was. Voor elk gen zijn die SNPs gekozen die het hele gen taggen. Verder gebruikten we in alle studies dezelfde strategie. Allereerst testten we alle SNPs in een eerste (identificatie) cohort. Zoals al eerder genoemd (in hoofdstuk 4); een significante genetische associatie in één cohort is niet voldoende om aan te tonen dat een genetische associatie ook een ware associatie is. Voor meer overtuigendere data heb je minimaal replicatie nodig en nog overtuigender is data dat de associatie op functioneel niveau onderschrijft. De volgende stap was daarom ook om de SNPs die significant geassocieerd waren met de toename van radiologische gewrichtsschade te testen in een ander (replicatie) cohort. Wanneer een SNP ook significant geassocieerd was met radiologische gewrichtsschade in het replicatie cohort, was de kans op een fout-positieve associatie verkleind. In de sommige gevallen hebben we de associatie nog kunnen bevestigen met functionele data.

In **hoofstuk 5** onderzochten we de associatie van genetische varianten in *IL15* met de snelheid van toename in radiologische gewrichtsschade in RA. Verschillende studies laten zien dat IL-15 een rol speelt in het onderhouden van inflammatie en beïnvloeden van

osteoclastogenesis. Bij patiënten met RA, zijn de IL-15 levels in het serum, het synovium en in het beenmerg verhoogd. Verder zijn IL-15 levels sterk gecorreleerd aan ziekte activiteit. Daarom onderzochten wij of genetische varianten in *IL-15* geassocieerd zijn met de ernst van gewrichtsschade in RA. Vijf SNPs waren significant geassocieerd met de snelheid van radiologische gewrichtsschade in de eerste fase (Leiden EAC) en werden dan ook 'gegenotypeerd' in drie andere cohorten (Groningen, Lund (Sw) en Sheffield (UK)). Onafhankelijke replicatie werd niet behaald, waarschijnlijk door het ontbreken van genoeg power in de replicatie cohorten. Maar een meta-analysis van alle vier de cohorten resulteerde in een significant resultaat voor alle vier de SNPs (rs7667746, rs7665842, rs4371699, rs6821171). Deze SNPs bleven significant na correctie voor 'multiple testing'. Dit suggereert dat deze vier genetische varianten in *IL15* geassocieerd zijn met toename van radiologische gewrichtsschade in RA. Verdere studies zouden nog moeten aantonen of de aanwezigheid van deze varianten ook daadwerkelijk leidt tot een verschil op functioneel niveau van IL15.

In **hoofdstuk 6** keken we naar de associatie tussen *IL4* en *IL4R* tagging SNPs en de snelheid van radiologische gewrichtsschade in RA. Eerdere studies lieten zien dat IL-4 een anti-inflammatoir en anti-osteoclastisch effect heeft. Het effect van IL-4 wordt voornamelijk gemedieerd door deIL-4 receptor alpha keten (IL4-Ralpha). Er zijn al verschillende genetische studies naar *IL4* en *IL4R* en gewrichtsschade gedaan, maar geen van de varianten die zijn gevonden zijn gerepliceerd. Daarom hebben wij een multi-cohort kandidaat studie gedaan. In de eerste fase (Leiden EAC) waren geen van de *IL4* SNPs en zeven van de *IL4R* SNPs significant geassocieerd met de snelheid van radiologische gewrichtsschade. Deze zeven significante SNPs werden geanalyseerd in vier (Groningen, Lund (Sw), Sheffiled (UK), NARAC (US)) of zes (Groningen, Lund (Sw), Sheffield (UK), NARAC (US), Wichita (US), NDB (US)) andere cohorten (afhankelijk van de beschikbaarheid van de genotypes van de verschillende SNPs in de cohorten). In deze kandidaat gen studie waren meer cohorten beschikbaar in de tweede fase. Daarom konden we nu wel onafhankelijke replicatie bewerkstelligen, die bestond uit een meta-analyse van alleen de extra cohorten in de tweede fase. In de replicatie fase waren twee SNPs in het *IL4R* gen significant geassocieerd met de snelheid van radiologische gewrichtsschade. Eén van deze twee SNPs bleef ook significant na correctie voor 'multiple testing'. Deze SNP (rs1119132) was recessief geassocieerd met radiologische gewrichtsschade toename. Dit betekent dat het dragen van beiden minor allelen resulteert in meer ernstige gewrichtsschade toename en dat maar een klein deel van de RA patiënten deze homozygote recessieve variant zal dragen. Desondanks vonden we zowel onafhankelijke replicatie in sommige replicatie cohorten als in de meta-analyse van de zes replicatie cohorten. Concluderend, identificeerden en repliceerden we een genetische variant in *IL4R*, die geassocieerd is met radiologische gewrichtsschade in RA. Verdere studies naar IL-4R op functioneel niveau zijn nodig om deze bevinding te bevestigen en beter te begrijpen wat de rol is van deze variant in de pathogenese van RA.

In **hoofdstuk 7** onderzochten we Granzyme B (*GZMB*) als kandidaat gen voor radiologische gewrichtsschade in RA. GZMB is een serine protease dat in lytische granules van 'natural killer'(NK) cellen en cytotoxische T lymfocyten zit en cel dood kan induceren. Eerdere studies suggereerden al een rol voor GZMB in RA gewrichtsdestruktie, vanwege de toename van GZMB-positieve cellen in synovium en tussen chondrocyten op de plek van een pannus laesie. Daarom onderzochten wij of er genetische varianten in *GZMB* geassocieerd zijn met toename van radiologische gewrichtsschade. Twee SNPs waren significant geassocieerd met radiologische gewrichtsschade in de eerste fase (Leiden EAC). Deze twee SNPs waren sterk gecorreleerd en daarom werd alleen de sterkste SNP (rs8192916) getest in de tweede fase. Omdat de data in de tweede fase onvoldoende was voor onafhankelijke replicatie, werd deze SNP getest in een meta-analyse van alle vier de datasets (Leiden EAC, Groningen, Lund (Sw) en Sheffield (UK)). Dit resulteerde in een significante associatie met radiologische gewrichtsschade. Deze associatie bleef ook significant na correctie voor 'multiple testing'. De minor allel van deze SNP was geassocieerd met een hogere snelheid van gewrichtsschade. Ook de associatie tussen RNA expressie en de genomische regio van *GZMB* werd geanalyseerd. Dit resulteerde in een hogere expressie van GZMB RNA bij aanwezigheid van de minor allel van deze SNP. Geconcludeerd kan worden dat dit suggereert dat deze genetische variant in *GZMB* geassocieerd is met de toename van radiologische gewrichtsschade en RNA expressie in het bloed in RA.

In **hoofdstuk 8** evalueerden we de voorspellende waarde van serum pyridinoline (PYD) levels in gewrichtsdestructie. PYD is een belangrijke schakel in collageen van kraakbeen, bot en synovium. Eerdere studies lieten zien dat PYD levels verhoogd zijn in RA patiënten vergeleken met gezonde personen en dat PYD levels verhoogd zijn in geval van actieve of ernstige ziekte. De meeste eerdere studies zijn uitgevoerd met urine PYD levels. Grote longitudinale studies met serum PYD levels en radiologische gewrichtsschade in RA zijn er niet. Wij evalueerden de voorspellende waarde van serum PYD voor toekomstige gewrichtsschade, zowel op baseline voor lange termijn voorspelling als gedurende de ziekte voor korte termijn voorspelling. Evaluatie van de baseline PYD serum levels toonde aan dat de baseline levels significant geassocieerd zijn met de snelheid van radiologische gewrichtsschade over zeven jaar en ook onafhankelijk van bekende risico factoren voor gewrichtsschade, zoals; leeftijd, geslacht, behandeling, ACPA status, CRP level, BMI en roken. Ook waren de PYD levels gedurende follow-up significant geassocieerd met de snelheid van radiologische gewrichtsschade voor het eerst volgende jaar en ook onafhankelijk van bekende risico factoren. Ook al vonden we dat een hoog PYD serum level een onafhankelijke risico factor was, de accuraatheid (gemeten doormiddel van de AUC) van PYD level als voorspeller voor de snelheid van radiologische gewrichtsschade was matig. Daarom kan geconcludeerd worden dat een verhoogd PYD serum level, zowel op baseline als gedurende de ziekte, geassocieerd is met een hogere snelheid van radiologische gewrichtsschade gedurende het volgende jaar/de volgende jaren, maar de voorspellende

accuraatheid als een losse voorspeller is matig. Daarom kan het belangrijk zijn om serum PYD toe te voegen aan een voorspel model met meerdere voorspellers om radiologische gewrichtsschade in RA patiënten te voorspellen.

DEEL III: MAGNETIC RESONANCE IMAGING VAN PATIËNTEN MET ARTRALGIE EN VROEGE ARTRITIS

Magnetic Resonance Imaging (MRI) wordt steeds belangrijker in het onderzoek naar RA. De weergave van hoog anatomisch detail samen met de afbeelding van inflammatie maken MRI een interessante nieuwe modaliteit bij het onderzoek naar vroege detectie van RA. Naast de detectie van inflammatie in de gewrichten, is inflammatie in het bot zichtbaar en van potentiële meerwaarde ten opzichte van conventioneel onderzoek en echografie. In dit deel onderzochten we diverse MR parameters in de (deels klinisch onzichtbare) vroege ziekte fases van RA. Verder onderzochten we of MRI RA patiënten kan onderscheiden van andere patiënten met vroege artritis.

Van eerdere studies weten we dat ACPA, RF en acute fase eiwitten al jaren voor de eerste symptomen van RA tot uiting komen, aanwezig zijn. Dit suggereert dat er een pre-klinische fase in RA bestaat. Het was alleen nog onduidelijk of er ook lokale inflammatie in de gewrichten plaats vindt in deze pre-klinische fase. Een MRI studie van knie gewrichten van 13 ACPA positieve artralgie patiënten liet geen subklinische inflammatie zien. Maar hoogst waarschijnlijk begint RA niet in de knie gewrichten en dus laat dit de vraag open of er lokale inflammatie aanwezig is in de pre-klinische fase van ACPA positieve RA. In **hoofdstuk 9** onderzochten we de kleine gewrichten van 21 ACPA positieve artralgie patiënten op lokale subklinische inflammatie. De inflammatie gedetecteerd met MRI werd vergeleken tussen kleine gewrichten van controles (n=19)(zonder gewrichtsklachten), kleine gewrichten van ACPA positieve artralgie patiënten en kleine gewrichten van ACPA positieve RA patiënten (n=22). Deze studie liet meer inflammatie op MRI in de PIP/MCP en pols gewrichten zien in ACPA positieve artralgie patiënten in vergelijking tot controles zonder gewrichtsklachten en minder dan in de gewrichten van ACPA positieve RA patiënten. Deze data suggereren dat lokale subklinische inflammatie aanwezig is in ACPA positieve artralgie patiënten. Waarschijnlijk is er al een inflammatie proces gaande in de kleine gewrichten, voordat deze gewrichten klinisch ontstoken zijn en opgemerkt worden als gezwollen gewrichten bij lichamelijk onderzoek.

De volgende vraag was of inflammatie in de gewrichten van artritis patiënten, die gedetecteerd wordt met MRI, overeenkomt met inflammatie die gedetecteerd wordt bij lichamelijk onderzoek. In **hoofdstuk 10** onderzochten we de associatie en congruentie tussen inflammatie van de kleine gewrichten gemeten met MRI en door middel van lichamelijk onderzoek. We onderzochten 1.790 gewrichten van 179 patiënten van de Leiden

EAC. In deze gewrichten waren zowel synovitis als tenosynovitis op MRI onafhankelijk geassocieerd met klinische zwelling, in tegenstelling tot beenmerg oedeem (BME). In het merendeel (~90%) van de gezwollen MCP pols gewrichten was enige inflammatie te zien op MRI. In 27% van de niet gezwollen MCP gewrichten en 66% van de niet gezwollen polsen was enige inflammatie te zien. Daar tegenover staat dat van alle MCP, pols en MTP gewrichten met enige inflammatie op MRI, respectievelijk 64%, 61% en 77%, niet gezwollen waren. BME, zowel kleine als grotere laesies, kwam vaak voor in klinisch niet gezwollen gewrichten. Bovendien vonden we soortgelijke resultaten voor pijnlijke gewrichten. Geconcludeerd kan worden dat inflammatie op MRI niet alleen aanwezig is in klinisch gezwollen gewrichten, maar ook in niet gezwollen gewrichten. Van belang is dat BME ook voorkomt in klinische niet gezwollen gewrichten. Van eerdere studies weten we dat BME een onafhankelijke voorspeller is voor radiologische gewrichtsschade. Echter of alle met MRI gedetecteerde BME klinische relevant is voor het verloop van de ziekte, is onbekend.

In **hoofdstuk 11** onderzochten we de relevantie van deze MRI gedetecteerde subklinische inflammatie in relatie tot radiologische gewrichtsschade gedurende het eerste jaar. De belangrijkste redenen voor het kiezen van radiologische gewrichtsschade als een uitkomst, is omdat dit veel gebruikt wordt in RA, het een objectieve maat is en omdat de score op joint niveau apart kan worden bekeken. Fysieke functie en ziekte activiteit zijn natuurlijk ook interessante uitkomstmaten, maar niet te gebruiken op gewrichtsniveau. Deze uitkomstmaten kunnen wel een belangrijke rol spelen bij het vertalen naar de klinische praktijk in de toekomst. In deze studie probeerden we allereerst te repliceren wat anderen al vonden, om er zeker van te zijn dat deze data zich het zelfde gedragen en dat dus de vooraf bedachte aannames ook van toepassing zijn op deze data. Wij vonden inderdaad ook dat BME, synovitis en tenosynovitis geassocieerd zijn met radiologische gewrichtsschade, onafhankelijk van bekende risico factoren. Daarna onderzochten we de relevantie van de subklinische inflammatie. Ongeveer 26% van alle niet gezwollen gewrichten had subklinische inflammatie. Radiologische gewrichtsschade was aanwezig in 4% van alle niet gezwollen gewrichten met subklinische inflammatie, in vergelijking tot 1% van alle niet gezwollen gewrichten zonder subklinische inflammatie. Soortgelijke bevindingen vonden we voor de inflammatie parameters apart; BME 7% versus 1%, synovitis 5% versus 1% en tenosynovitis 4% versus 1%. Daarom kan worden geconcludeerd dat, ook al komt radiologische gewrichtsschade bijna niet voor, gewrichten met subklinische inflammatie wel een verhoogde kans op radiologische gewrichtsschade hebben binnen het eerste jaar. Dit laat de relevantie zien van MRI gedetecteerde subklinische inflammatie. Vooralsnog is het lastig te vertalen in de klinische praktijk, omdat deze analyses op gewrichtsniveau zijn gedaan. Een reumatoloog behandelt een patiënt en niet een gewricht en bovendien waren de gewrichten met subklinische inflammatie verdeeld over 91% van de patiënten. Daarom

kan deze studie vooralsnog vooral iets bijdragen aan het beter begrijpen van de connectie tussen inflammatie en structurele schade in de vroege ziekte.

De EULAR taskforce heeft recent gesuggereerd dat MRI kan helpen bij onzekerheid over de diagnose RA. Omdat deze aanbevelingen neigen naar het gebruik van MRI in de dagelijkse praktijk, zijn goede studies naar de waarde van MRI nodig. Tot nog toe is er geen grote studie gedaan, waarin wordt gekeken naar de accuraatheid van MRI voor differentiatie van vroege RA van andere vroege artritis patiënten. In hoofdstuk 12 hebben we een grote cross-sectionele studie gedaan, om te bepalen of patiënten die klinisch geclassificeerd worden als RA andere MRI eigenschappen hebben dan patiënten met andere diagnoses. We vonden dat tussen alle patiënten met vroege artritis, RA patiënten een hogere score voor synovitis, tenosynovitis en BME hadden ten opzichte van de patiënten zonder RA. Ook al was de negatief voorspellende waarde van alle MRI inflammatie parameters voor RA goed (>0,80), de positief voorspellende waarde voor RA was laag (<0,50). Voor alle MRI parameters waren de AUCs <0,70. Ook hebben we gekeken naar ACPA positieve en negatieve UA en RA patiënten en vonden dat de ACPA positieve patiënten meer BME hadden dan de ACPA negatieve patiënten. Van eerdere studies weten we dat BME een sterke onafhankelijke voorspeller is voor toename van gewrichtsschade en dat komt overeen met het feit dat ACPA positieve RA een ernstiger ziektebeeld kent. Verder hebben we patiënten die voldeden aan de 2010 criteria voor RA vergeleken met patiënten die voldeden aan de 1987 criteria voor RA. Dit deden we door de patiënten in vier groepen in te delen; of ze voldeden aan beide criteria sets, of aan één of aan geen van beiden. We vonden dat patiënten die aan de 2010 criteria voldeden maar niet aan de 1987 criteria minder synovitis hadden. Dit suggereert dat patiënten die alleen aan de 2010 criteria voldoen een mildere ziekte hebben, wat overeenkomt met resultaten van eerdere studies. Geconcludeerd kan worden dat ook al hebben RA patiënten hogere inflammatie scores op MRI en ACPA positieve patiënten meer BME, de ernst van de MRI inflammatie kan niet accuraat RA patiënten differentiëren tussen andere vroege artritis patiënten. Een interessante vraag zou zijn of MRI een toegevoegde waarde kan hebben in een groep patiënten die UA hebben en RA gaan ontwikkelen. Hiervoor zijn grote longitudinale studies nodig die kunnen laten zien of MRI een toegevoegde waarde kan hebben in het classificeren van patiënten die klinisch ongeclassificeerd zijn.

DEEL IV: MRI SCAN PROTOCOL HERZIEN

In dit deel hebben we het MRI scan protocol herzien. De OMERACT MRI in RA werkgroep ontwikkelde en valideerde een semi-kwantitatieve scoringsmethode systeem (RAMRIS). In het protocol wordt aanbevolen om minimaal de volgende vijf sequenties te maken: in twee richtingen (coronaal en axiaal) T1-gewogen sequenties voor en na intraveneus

(Gadolinium-Chelaat) contrast en een T2-gewogen vet onderdrukte sequentie in één richting. Deze set van sequenties neemt veel tijd in beslag en is daarom zeer kostbaar en niet erg patiëntvriendelijk. Het verkorten van dit protocol zou kunnen leiden tot een betere kosten-effectiviteit verhouding en zou patiënt vriendelijker zijn.

Op MRI is BME zichtbaar door plaatselijke toename van watergehalte in het beenmerg, door gedeeltelijk of geheel vervangen van het beenmerg vet. Daarom is de signaalintensiteit van BME laag op T1-gewogen sequenties en hoog op T2 gewogen vet onderdrukte sequenties. BME licht ook op met intraveneus gadolinium op T1 gewogen vet onderdrukte sequenties en dit ziet er bijna net zo uit als op T2 gewogen vet onderdrukte sequenties.

In **hoofdstuk 13** onderzochten we of voor de beoordeling van BME de T1 gewogen post-gadolinium sequenties (T1Gd) de T2 gewogen sequenties (T2) zou kunnen vervangen. We zagen dat totale BME scores niet verschilden tussen de twee sequenties (T1Gd en T2). Intrareader ICCs tussen de scores op T2 en T1Gd sequentie waren allemaal uitstekend (0,80-0,99). De sensitiviteit en specificiteit van BME op T1Gd vergeleken met T2 was hoog. Interreader ICCs waren uitstekend, ongeacht de beelden set die werd gebruikt (allemaal>0,83).

Geconcludeerd kan worden dat T1Gd sequenties dezelfde scores voor BME opleverden als de standaard T2 sequenties. Daarom zou voor het scoren volgens de RAMRIS een kort protocol met T1 en T1 vet onderdrukte post- gadolinium sequenties voldoende moeten zijn. Dit zou dan resulteren in 20% reductie van de scan tijd. Daarnaast zijn T2 gewogen sequenties gevoeliger voor artefacten dan de T1 gewogen sequenties door magnetisch veld heterogeniteit en beweging. Vooral wanneer in één sessie meerdere gewrichten afgebeeld moeten worden, zorgt een verkorting van de scan tijd in combinatie met robuustere sequenties voor een kleinere kans dat de MRI beelden mislukken.

Volgens het RAMRIS protocol en het extra protocol voor tenosynovitis score door Haavardsholm, is intraveneus contrast toediening nodig om synovitis en tenosynovitis te scoren op T1 gewogen post-contrast beelden. Synovitis en tenosynovitis geven echter zowel op T1 gewogen post-contrast beelden als op T2 gewogen beelden een hoog signaal. In **hoofdstuk 14** onderzochten we of intraveneuze contrast toediening weggelaten kan worden in het MRI scan protocol, zodat de scan tijd kan worden verkort, de kosten omlaag gaan en de invasiviteit voor de patiënt afneemt.

We vergeleken de scores voor synovitis en tenosynovitis op T2 gewogen vet onderdrukte sequenties(T2) met de scores op de volgens het protocol T1 gewogen sequenties na toediening van contrast (T1Gd). Op individueel gewricht/pees niveau was de sensitiviteit om synovitis op sequenties zonder contrast te detecteren 72% en 91% voor beide lezers en de specificiteit 52% en 81%, vergeleken met sequenties na contrast. Voor tenosynovitis was de sensitiviteit 54% en 67% en de specificiteit 88% en 91%. Een review van de literatuur liet zien dat bij het scoren van beelden zonder contrast, synovitis een hoge sensitiviteit en lage specificiteit heeft en tenosynovitis een lage sensitiviteit en hoge specificiteit. Een

verklaring voor de hoge sensitiviteit en lage specificiteit voor het scoren van synovitis op beelden zonder contrast, zou kunnen zijn dat op T2 gewogen sequenties hypervasculaire synoviale woekering en vocht in het gewricht niet goed te onderscheiden zijn. Geconcludeerd kan worden dat bij het weglaten van de toediening van contrast de specificiteit van synovitis en de sensitiviteit van tenosynovitis omlaag gaan en dat onderstreept dat intraveneus contrast toedienen essentieel blijkt voor een optimale beoordeling

Een extra opmerking over het vergelijken van de scores op de verschillende MRI sequenties is hierbij wel op zijn plaats. Het scoren van BME, synovitis en tenosynovitis volgens de RAMRIS methode houdt niet altijd in dat de inflammatie die wordt gezien ook daadwerkelijk pathologisch is. MRI staat bekend als een erg gevoelig meetinstrument en daarom kan aangenomen worden dat lage scores van sommige parameters ook fysiologisch normale variaties kunnen zijn. Daarom is het ook niet automatisch zo dat de resultaten van het scoren op andere sequenties, dan volgens de RAMRIS methode, slechtere resultaten opleveren. Belangrijker is wat de klinische consequentie van de score is. Ook is het belangrijk om te weten wat de sensitiviteit voor verandering is, wat een belangrijke maat in klinische trials kan zijn.

In **hoofdstuk 15** onderzochten we het mogelijke effect van mechanische belasting van de voorvoet op MRI bevindingen. Omdat het dragen van hoge hakken de druk naar de voorvoet en de kopjes van de metatarsalia verplaatst, veronderstelden wij dat dit zou kunnen resulteren in afwijkingen aan het bot of de weke delen. We deden een 'single-blind cross-over studie' om dit te bepalen. Op de eerste dag droegen de proefpersonen platte schoenen en op de tweede dag droegen ze hoge hakken. De MRI's werden op beide dagen twee keer per dag gemaakt; aan het begin en aan het einde van de dag. Er werden geen BME of diepe weke delen afwijkingen gezien, veroorzaakt door het dragen van hoge hakken voor een dag. De enige afwijking was op alle MRI beelden aan het einde van dag twee (na het dragen van hoge hakken) te zien: plantair subcutaan oedeem, altijd in de mediale voorvoet proximaal van de MTP gewrichten. Deze data laten zien dat het dragen van hoge hakken niet geassocieerd is met korte termijn structurele afwijkingen. De lange termijn effecten van het dragen van hoge hakken blijft echter onduidelijk. Deze resultaten zijn relevant bij het interpreteren van MRI beelden van de voorvoet van reumatische en orthopedische patiënten.

ALGEMENE CONCLUSIE

In dit proefschrift probeerden we de volgende vragen te beantwoorden:

1) Hoe kunnen we patiënten/personen die RA gaan ontwikkelen onderscheiden van de patiënten/personen die geen RA ontwikkelen? Wat zijn voorspellende factoren voor de ontwikkeling van RA?

2) Wat is de prognose van een RA patiënt? Wat zijn voorspellende factoren voor het ziekte verloop van RA?

1) In hoofdstuk 12 vonden we dat RA patiënten hogere MRI inflammatie scores hadden dan andere vroege artritis patiënten. Helaas was de accuraatheid om RA patiënten van andere vroege artritis patiënten te differentiëren laag. In de klinische praktijk worden patiënten eerst geclassificeerd op basis van klinische en serologische bevindingen. De overgebleven groep bestaande uit UA patiënten kunnen mogelijk onderscheiden worden met MRI bevindingen. In hoofdstuk 2 en 3 keken we naar de differentiatie van deze UA patiënten. We zagen dat zelfs na het toepassen van de meer gevoelige 2010 criteria voor RA, nog 24% RA ontwikkelden gedurende het eerste jaar. Dit laat zien dat de 2010 criteria tekort schiet bij het classificeren van RA op baseline. We probeerden voorspellende factoren te vinden voor UA patiënten die RA ontwikkelen. Helaas konden ACPA status en het 'Van der Helm voorspel model' deze patiënten niet differentiëren.

In hoofdstuk 9 deden we onderzoek met patiënten in een nog eerdere fase dan UA; in ACPA positieve artralgie patiënten. We zagen in deze vroege fase van de ziekte al lokale subklinische inflammatie op MRI. Dit bevestigt de aanwezigheid van een pre-klinische fase van RA en het belang van MRI daarin. In deze fase van de ziekte zou MRI kunnen helpen in het voorspellen welke patiënten RA zullen ontwikkelen.

2) In hoofdstuk 10 en 11 zagen we met behulp van MRI subklinische inflammatie in de klinisch niet gezwollen gewrichten in vroege artritis patiënten. Om de relevantie van deze subklinische inflammatie in de klinisch niet gezwollen gewrichten te onderzoeken, keken we naar radiologische toename van gewrichtsschade na één jaar. We vonden dat gewrichten met subklinische inflammatie een verhoogd risico hadden op radiologische gewrichtsschade toename. Dit suggereert dat MRI gedetecteerde subklinische inflammatie relevant is en kan voorspellen voor een ernstiger ziekte beloop.

In hoofdstuk 5 tot 7 vonden we vier IL15, één IL4R en één GZMB SNPs die geassocieerd zijn met een ernstiger ziekte beloop. In hoofdstuk 8 vonden we ook een serologische marker, pyridinoline, die geassocieerd is met een ernstiger ziekte beloop.

Ten slotte onderzochten we in hoofdstuk 13 tot 15 het huidige MRI scan protocol, met als doel; dit protocol efficiënter en accurater te maken. We vonden dat beenmerg oedeem (BME) ook gescoord kan worden op een T1 gewogen sequentie na contrast toediening en daarom kan nu het scan protocol verkort worden door de T2 gewogen sequenties weg te laten. Ook vonden we dat sequenties na toediening van contrast essentieel zijn om synovitis en tenosynovitis goed te kunnen scoren. Ook hebben we laten zien dat het dragen van hoge hakken niet zorgt voor structurele afwijkingen op de korte termijn.

TOEKOMST PERSPECTIEVEN/UITDAGINGEN

Gedurende de afgelopen decennia is de behandeling van RA zeer effectief geworden, wat heeft geresulteerd in een minder ernstige uitkomst van RA, met zeer weinig radiologische gewrichtsschade toename. Verder hebben we uit eerdere onderzoeken geleerd dat vroege interventie het natuurlijk ziekte beloop van RA kan stoppen. Daarom zou de behandeling van RA zich in de toekomst nog meer moeten richten op het de **vroege detectie van RA patiënten**, om hen vervolgens te behandelen of mogelijk eerst goed te monitoren in het geval van heel vroege RA. Daarom zou het onderzoek gericht moeten zijn op het identificeren van voorspellende factoren voor het ontwikkelen van RA. Dit is al veel gedaan in UA patiënten, maar de kansen liggen in de eerdere fases van de ziekte. We weten van eerdere studies dat anti-lichamen en acute fase eiwitten al jaren aanwezig zijn, voordat de eerste symptomen zich openbaren. Dit suggereert dat er een pre-klinische fase bestaat in RA. Het lastige is dat voorspellende factoren voor RA ontwikkeling in een heel vroege fase van de ziekte erg specifiek kunnen zijn, maar de uitdaging zit hem in om ook sensitief te blijven. Een voorbeeld in artralgie patiënten en dan vooral ACPA en/of RF positieve artralgie patiënten is dat zij een verhoogd risico hebben om RA te ontwikkelen, maar als deze patiënten ook artritis hebben (UA patiënten) is de kans op RA nog veel hoger. Hoe verder je af zit van het daadwerkelijke RA fenotype, hoe kleiner de kans op ontwikkeling van RA en hoe moeilijker het is om te differentiëren.

Een andere belangrijke pijler in RA onderzoek zou **'personalized medicine'** moeten zijn. De ene RA patiënt is de ander niet. Allereerst zou het onderzoek zich meer moeten richten op het voorspellen van het ziekte beloop in de individuele patiënt. Ten tweede, zou het onderzoek zich moeten richten op het voorspellen van de effectiviteit van een behandeling (en de bijwerkingen) in de individuele patiënt. Deze twee zouden dan gecombineerd moeten worden om een persoonlijke behandelkeuze te maken in iedere RA patiënt. Ik verwacht dat er in de nabije toekomst handige tools zullen zijn om te berekenen welke behandel stap genomen moet worden in iedere patiënt, gebaseerd op de voorspelling van zijn ziekte beloop en effectiviteit van elke behandeling.

Een ander belangrijk onderwerp in RA onderzoek is **MRI**. MRI is nu al een belangrijk onderwerp in RA onderzoek, maar zal mogelijk in de toekomst alleen nog maar belangrijker worden. MRI is een sensitief instrument om (sub)klinische inflammatie te meten. MRI is het één van de weinige niet invasieve instrumenten waarmee BME, een sterke voorspeller voor progressieve ziekte, kan worden gemeten. MRI zou een belangrijk instrument kunnen worden in het maken van behandel keuzes en het voorspellen van de prognose van de patiënt. In klinische trials zal MRI steeds belangrijker worden voor het meten van de effectiviteit van ziekte onderdrukkende behandelingen, op het verloop van synovitis en beenmerg oedeem. Zo zijn er al studies die hebben aangetoond dat er sprake kan zijn van subklinische inflammatie op MRI in patiënten in klinische remissie.

Om al deze uitdagingen in het onderzoek naar RA aan te gaan, is er nog veel onderzoek nodig en wie weet komt het ooit zo ver dat de ziekte RA niet meer bestaat.

Dankwoord

Op deze plaats wil ik iedereen bedanken die heeft bijgedragen aan de totstandkoming van dit proefschrift.

Allereerst wil ik mijn promotor en copromotoren bedanken. Professor Huizinga, beste Tom, bedankt dat ik deel mocht uitmaken van deze afdeling. Ik heb mogen ervaren hoe bijzonder deze afdeling is, met de vele goede samenwerkingsverbanden die er zijn zowel binnen als buiten de afdeling.

Dr. Van der Helm-van Mil, beste Annette, jou wil ik bedanken voor de vele goede wetenschappelijke discussies, je kritische blik en de mogelijkheden en kansen die je mij hebt gegeven. Ik heb veel bewondering voor jouw passie voor onderzoek.

Dr. Reijnierse, beste Monique, graag wil ik jou bedanken voor de andere kijk op resultaten, alle inzichten in de anatomie van de hand en voet gewrichten, maar ook heb ik jouw persoonlijke betrokkenheid gewaardeerd.

Een groot deel van dit proefschrift is gebaseerd op data uit de EAC. Daarom ben ik ook veel dank verschuldigd aan alle patiënten die hebben deelgenomen aan dit cohort, alsmede alle collega's die hebben meegewerkt aan het in stand houden en uitbreiden van dit cohort.

Tijdens mijn promotie heb ik geleerd dat onderzoek doen niet mogelijk is zonder goede samenwerkingsverbanden.

Daarom wil ik ook graag de afdeling radiologie bedanken. Prof. Bloem, hartelijk dank voor de gegeven mogelijkheden en de samenwerking. Ook wil ik graag iedereen bedanken die direct of indirect betrokken is geweest bij het maken van de vele MRI's. In het bijzonder Wouter Stomp, voor de samenwerking en de vele uren (leren) scoren van de MRI beelden.

Voor het verwerken van alle data is een goed datamanagement team onmisbaar. Jozé en Cedric hartelijk dank voor het verwerken, beheren en aanleveren van de data.

Ook iedereen van het lab wil ik bedanken voor alle hulp. Prof. Rene Toes, veel dank voor je kritische blik op de statistische testen die we toepasten. Nivine en Linda bedankt voor het altijd weer klaar staan voor het doen van bepalingen.

Graag bedank ik hierbij ook mensen die hebben geholpen bij de statistiek en methodologie. Prof. Saskia le Cessie, dank voor de altijd duidelijke uitleg van de meest ingewikkelde statistische methoden. Prof Desirée van der Heijde, bedankt voor je opbouwende kritiek en methodologische inzicht.

Ook wil ik graag Hughine, Joyce, Nancy en Hanny van het secretariaat bedanken voor alle praktische, maar ook persoonlijke steun.

I would also like to thank some people from outside the LUMC. A lot of the research would not be of any value without replication in appropriate replication cohorts. That is why I would like to thank the group of Reade in Amsterdam (prof. Dirk-Jan van Schaardenburg and drs. Karin Britsemmer) and the group in Birmingham, United Kingdom (prof. Karim Raza, dr. Abhishek Abhishek and dr. Andrew Filer) for the pleasant collaboration. Also many thanks to the groups from Lund, Sweden (prof. T. Saxne), Sheffield, United Kingdom (prof. G. Wilson), Groningen (dr. E. Brouwer) and the American group (prof. P. Gregersen) for their excellent collaboration in the genetic studies we performed.

En natuurlijk dank ik mijn directe collega's op 'eiland Annette' voor de goede wetenschappelijke discussies, het luisterend oor en de fijne samenwerking. Rachel, wat heb jij mij veel geleerd over statistiek, onderzoek doen en natuurlijk alle snel-toetsen. Diederik dank voor de goede discussies die we hadden. Hanna, Wouter Nieuwenhuis, Lukas jullie dank ik ook voor de goede aanvulling van het team. Dank aan alle mensen in kamer C1-46: Emilia, Iris, Jessica Meijs, Sacha, Nina, Victoria, Angga, Wing-Yee, Rosaline, Manouk en Cesar. Dank jullie voor de goede sfeer in deze drukke kamer, daar waar een goede harmonie heerste tussen werk en gezelligheid. Muchas gracias!

Natuurlijk ook de mensen uit kamer C1-45: Willemien, Annemiek, Rani, Badelog, Lotte, Kirsten, Gülsah, Marianne, Linda, Wendy, Pauline. Dank voor de al dan niet wetenschappelijke discussies in de koffiecorner.

Tijdens het promoveren is het belangrijk dat je onderzoeksomgeving goed is, maar de mensen daaromheen zijn mogelijk nog belangrijker om het uiteindelijke resultaat te kunnen behalen. Daarom wil ik allereerst alle Vrienden en vriendinnen van Lucht-en Ruimtevaart techniek (VvLR) bedanken. Zeer dierbare vrienden, Ik ben blij dat we nog altijd zo' n hechte vriendengroep zijn. Dank voor de noodzakelijke afleiding tijdens mijn promotie tijd.

Lieve meiden van jong dames 2 van Qui Vive. Dank voor de fijne opname in het team en de nodige afleiding.

Waarde paranimfen, jullie zijn op twee totaal verschillende manieren betrokken bij het tot stand komen van dit proefschrift. Jessica, dank voor alle ervaringen die ik met jou kon delen tijdens ons bezoek aan Oslo, de vele congressen en natuurlijk elke dag op ons eiland. Dank voor je luisterend oor, je wijze woorden op de goede momenten en je gastvrijheid. Erika, dank voor je interesse, je relativeringsvermogen en natuurlijk de gezelligheid. Ik vind het geweldig dat jullie op deze bijzondere dag aan mijn zijde willen staan.

Lieve mama, dank voor de belangrijke steun op momenten dat ik dat het hardst nodig had. Altijd heb je mij verzekerd dat ik meer kon dan ik zelf soms dacht. Dat papa mijn promotie niet meer mee kan maken is een groot gemis. Maar wat graag had ik hem trots willen zien op deze dag.

Tenslotte aller liefste Alex, dank voor je steun en liefde. Bedankt voor je geduld en een luisterend oor op de momenten dat ik het nodig had. Ik kijk uit naar onze toekomst samen.

Curriculum vitae

Annemarie Krabben werd op 12 oktober 1983 geboren in Rotterdam. Zij woonde de eerste 10 jaar van haar leven in Berkel en Rodenrijs. Daarna is zij verhuisd naar Zoetermeer waar zij in 2002 haar VWO diploma behaalde aan het Alfrink college.

In september 2002 begon zij met de studie biomedische wetenschappen aan de Universiteit van Leiden. In 2005 deed zij tijdens haar bachelor stage onderzoek naar een kandidaat gen als mogelijke risico factor voor borstkanker bij de afdeling Human Genetica in het LUMC. In hetzelfde jaar behaalde zij haar bachelor diploma biomedische wetenschappen.

In 2006 heeft zij voor haar master biomedische wetenschappen onderzoek gedaan naar een nieuw slaapmiddel in gezonde personen aan het CHDR (Center for Human Drug Research).

In 2006 startte zij met de studie geneeskunde, waarvan zij in 2008 haar doctoraal behaalde. Direct daarna ging zij verder met haar afstudeerstage en afstudeerthesis voor de master van haar studie biomedische wetenschappen bij de afdeling Klinische Farmacie en Toxicologie in het LUMC. Dit onderzoek had als onderwerp: Het effect van CYP2C9 polymorfismen en de tijd tot een stabiele dosis van sulfonylureum derivaten in diabetes type 2 patiënten in de huisartspraktijk. In 2009 behaalde zij haar Research Master Biomedical Sciences.

In 2009 startte zij met haar co-schappen voor haar studie geneeskunde en deed haar semi-arts stage bij de afdeling oncologie van het LUMC. In 2011 behaalde zij haar artsexamen; ook in dat jaar startte zij haar promotie onderzoek aan de afdeling reumatologie van het LUMC.

Momenteel is zij werkzaam als Study Responsible Physician voor de reumatologie in het Research Team van Medical Affairs Europe, bij Janssen Biologics B.V. te Leiden.

List of publications

Nieuwenhuis WP, **Krabben A**, Stomp W, Huizinga TW, van der Heijde D, Bloem JL, van der Helm-van Mil AH, Reijnierse M. Evaluating MRI-detected tenosynovitis of the hand and wrist in early arthritis. *Ann Rheum Dis.* Submitted.

Krabben A, Stomp W, Van Nies JA, Huizinga TW, Van der Heijde D, Bloem JL, Reijnierse M, Van der Helm-van Mil AH. MRI detected subclinical joint inflammation is associated with radiographic progression. *Ann Rheum Dis.* 2014 Jul 29. Epub ahead of print.

Krabben A, Huizinga TW, van der Helm-van Mil AH. Biomarkers for radiographic progression in rheumatoid arthritis. *Current Pharmaceutical Design.* 2014 Augustus 25. Epub ahead of print.

Stomp W, **Krabben A**, Van der Heijde D, Huizinga TW, Bloem JL, Ostergaard M, Van der Helm-van Mil AH, Reijnierse M. Aiming for a shorter scanning protocol in early arthritis MRI: can gadolinium administration be eliminated? *Eur Radiol.* Under review.

Stomp W, **Krabben A**, Van der Heijde D, Huizinga TW, Bloem JL, Van der Helm-van Mil AH, Reijnierse. Aiming for a shorter rheumatoid arthritis MRI protocol: can contrast-enhanced MRI replace T2 for the detection of bone marrow oedema? *Eur Radiol.* 2014 Oct;24 (10):2614-22.

Stomp W, **Krabben A**, Van der Heijde D, Huizinga TW, Bloem JL, Van der Helm-van Mil AH, Reijnierse M. Are rheumatoid arthritis patients discernible from other early arthritis patients using 1.5 extremity MRI?: a large cross-sectional study. *J Rheumatol.* 2014 Aug;41(8):1630-7.

van Nies JA, van Steenbergen HW, **Krabben A**, Stomp W, Huizinga TW, Reijnierse M, van der Helm-van Mil AH. Evaluating processes underlying the predictive value of baseline erosions for future radiological damage in early rheumatoid arthritis. *Ann Rheum Dis.* 2014 Jan 15. Epub ahead of print.

Krabben A, Stomp W, Huizinga TW, van der Heijde D, Bloem JL, Reijnierse M, van der Helm-van Mil AH. Concordance between inflammation at physical examination and on MRI in patients with early arthritis. *Ann Rheum Dis.* 2013 Dec 12. Epub ahead of print.

Stomp W, **Krabben A**, van der Helm-van Mil A, Reijnierse M. Effects of wearing high heels on the forefoot: an MRI evaluation. *Scand J Rheumatol.* 2014;43(1):80-1.

Krabben A, Wilson AG, de Rooy DP, Zhernakova A, Brouwer E, Lindqvist E, Saxne T, Stoeken G, van Nies JA, Knevel R, Huizinga TW, Toes R, Gregersen PK, van der Helm-van Mil AH. Association of genetic variants in the IL4 and IL4R genes with the severity of joint damage in rheumatoid arthritis: a study in seven cohorts. *Arthritis Rheum.* 2013 Dec;65(12):3051-7.

Krabben A, Stomp W, van der Heijde DM, van Nies JA, Bloem JL, Huizinga TW, Reijnierse M, van der Helm-van Mil AH. MRI of hand and foot joints of patients with anticitrullinated peptide antibody positive arthralgia without clinical arthritis. *Ann Rheum Dis.* 2013 Sep 1;72(9):1540-4.

Krabben A, Knevel R, Huizinga TW, Cavet G, van der Helm-van Mil AH. Serum pyridinoline levels and prediction of severity of joint destruction in rheumatoid arthritis. *J Rheumatol.* 2013 Aug;40(8):1303-6.

Knevel R, de Rooy DP, Zhernakova A, Gröndal G, **Krabben A**, Steinsson K, Wijmenga C, Cavet G, Toes RE, Huizinga TW, Gregersen PK, van der Helm-van Mil AH. Association of variants in IL2RA with progression of joint destruction in rheumatoid arthritis. *Arthritis Rheum.* 2013 Jul;65(7):1684-93.

Krabben A, Abhishek A, Britsemmer K, Filer A, Huizinga TW, Raza K, van Schaardenburg DJ, van der Helm-van Mil AH. Risk of rheumatoid arthritis development in patients with unclassified arthritis according to the 2010 ACR/EULAR criteria for rheumatoid arthritis. *Rheumatology (Oxford).* 2013 Jul;52(7):1265-70.

de Rooy DP, Yeremenko NG, Wilson AG, Knevel R, Lindqvist E, Saxne T, **Krabben A**, Leijsma MK, Daha NA, Tsonaka S, Zhernakova A, Houwing-Duistermaat JJ, Huizinga TW, Toes RE, Baeten DL, Brouwer E, van der Helm-van Mil AH. Genetic studies on components of the Wnt signalling pathway and the severity of joint destruction in rheumatoid arthritis. *Ann Rheum Dis.* 2013 May;72(5):769-75.

Maehlen MT, Provan SA, de Rooy DP, van der Helm-van Mil AH, **Krabben A**, Saxne T, Lindqvist E, Semb AG, Uhlig T, van der Heijde D, Mero IL, Olsen IC, Kvien TK, Lie BA. Associations between APOE genotypes and disease susceptibility, joint damage and lipid levels in patients with rheumatoid arthritis. *PLoS One.* 2013 Apr 17;8(4):e60970.

van Nies JA, **Krabben A**, Schoones JW, Huizinga TW, Kloppenburg M, van der Helm-van Mil AH. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis.* 2014 May;73(5):861-70.

Knevel R, **Krabben A**, Wilson AG, Brouwer E, Leijsma MK, Lindqvist E, de Rooy DP, Daha NA, van der Linden MP, Tsonaka S, Zhernakova A, Westra HJ, Franke L, Houwing-Duistermaat JJ, Toes RE, Huizinga TW, Saxne T, van der Helm-van Mil AH. A genetic variant in granzyme B is associated with progression of joint destruction in rheumatoid arthritis. *Arthritis Rheum.* 2013 Mar;65(3):582-9.

Knevel R, **Krabben A**, Brouwer E, Posthumus MD, Wilson AG, Lindqvist E, Saxne T, de Rooy D, Daha N, van der Linden MP, Stoeken G, van Toorn L, Koeleman B, Tsonaka R, Zhernakoza A, Houwing-Duistermaat JJ, Toes R, Huizinga TW, van der Helm-van Mil A. Genetic variants in IL15 associate with progression of joint destruction in rheumatoid arthritis: a multicohort study. *Ann Rheum Dis.* 2012 Oct;71(10):1651-7.

Krabben A, Huizinga TW, van der Helm-van Mil AH. Undifferentiated arthritis characteristics and outcomes when applying the 2010 and 1987 criteria for rheumatoid arthritis. *Ann Rheum Dis.* 2012 Feb;71(2):238-41.

Swen JJ, Wessels JA, **Krabben A**, Assendelft WJ, Guchelaar HJ. Effect of CYP2C9 polymorphisms on prescribed dose and time-to-stable dose of sulfonylureas in primary care patients with Type 2 diabetes mellitus. *Pharmacogenomics.* 2010 Nov;11(11):1517-23.