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The course of clinically suspect arthralgia and early rheumatoid arthritis : clinical features, imaging and genetics

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The course of Clinically Suspect Arthralgia and early Rheumatoid Arthritis

Clinical features, imaging and genetics

Hanna van Steenbergen
2016

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Clinical features, imaging and genetics

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1 General introduction

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic autoimmune disease that is characterised by persistent inflammation and destruction of the small joints [1]. The disease can result in significant morbidity with pain, loss of function and work disability and consequently high socio-economic costs [2–4]. It is the most common inflammatory arthritis with a worldwide prevalence of 0.5-1% and mostly affects middle-aged female (female male ratio 3:1), but it can occur at every age [1]. Based on data of Dutch general practitioners (NIVEL), the prevalence in the Netherlands was in 2013 1.3% [5].

The etiology of RA is largely unknown, but RA is considered to have an autoimmune origin because of the presence of autoreactive antibodies. These autoantibodies include rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) [1]. Especially ACPA has a high specificity for RA (ACPA is only present in 1% of the general population [6]) and can be detected in 50-60% of all early RA patients [7–9]. Although several pathophysiological mechanisms have been proposed, it is still unclear how RA-related autoantibodies exert their effects [10]. However, ACPA-positive and ACPA-negative RA are considered different disease entities with different underlying pathogeneses and disease courses [11]. Recently, novel autoantibody systems in RA have been identified, such as anti-carbamylated protein antibodies (anti-CarP).[12] These autoantibodies were present in both ACPA-positive and ACPA-negative patients, suggesting broad autoantibody production within RA and autoimmunity also in patients currently considered as seronegative.

In addition, more than 100 genetic risk factors for RA have been identified thus far [13]. Although the heritability of RA is reported to be up to 50% in both ACPA-positive and ACPA-negative RA [14], they have a different genetic background and most genetic risk factors are identified within ACPA-positive RA [11,15]. The most important genetic risk factor for ACPA-positive RA is located in the HLA-DRB1 region. The predisposing alleles share a similar amino acid sequence at positions 70-74 in the peptide-binding groove of the HLA-DRB1 molecule (the shared epitope (SE)). The SE hypothesis postulates that the SE motif itself may be directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide to T-cells [16]. Recently, a further refinement of the association of HLA with RA was proposed by making use of advanced statistical analyses [17]. Variations in HLA-DRB1 position 11 most strongly associated with development of both ACPA-positive [17] and ACPA-negative RA [18]; within ACPA-positive RA this association was independent of the SE status [17].

Furthermore, environmental factors might play a role in RA development. Many potential environmental risk factors have been suggested [19], but smoking is the only widely replicated environmental risk factor, especially for ACPA-positive RA in patients carrying HLA-SE alleles [20].

Therapeutic approaches for RA have changed considerably the last decades from

conservative step-up strategies to early and aggressive treat-to-target strategies with disease-modifying antirheumatic drugs (DMARDs) and biologics [21]. The importance of early treatment was set by observations that delay of treatment initiation was associated with a worse disease outcome, such as more severe joint damage and a lower chance on achieving remission [8,22,23]. The concept of the 'window of opportunity' proposed that there is an early phase in the disease in which the disease can be modified more successfully, presumably because the underlying disease processes are not yet fully matured [24]. The exact duration of this period is unknown, though it has been suggested that this period consist of 12 weeks and is a confined period [25]. Because treatment initiation in this early phase result in more beneficial long-term outcomes, the field of RA is moving into identifying RA in very early disease stages.

THE PHENOTYPE OF RA

A typical clinical presentation of RA consists of joint pain and swelling, morning stiffness and a symmetric polyarthritis of the small hand and foot joints. In addition, systemic symptoms such as fatigue and weight loss can be present. However, the presentation of RA may be considerably heterogeneous. In clinical practice, the diagnosis is made based on the judgment and expertise of the rheumatologist as no diagnostic test or diagnostic criteria for RA exist.

Classification criteria for RA have been developed to identify homogeneous groups of patients for enrolment in clinical studies, particularly trials. The 1987 ACR criteria for RA were designed to differentiate patients with established RA from patients with other types of rheumatic diseases (Table 1) [26]. These criteria included the items radiographic changes and rheumatoid nodules which are characteristic for advanced disease and classify mainly patients with established RA. With the increasing insights that early treatment initiation in RA is beneficial, clinical trials were designed to treat patients in more early disease phases. For this purpose, the 1987 criteria were inappropriate because of its poor sensitivity to classify patients with early RA.

The 2010 ACR/EULAR criteria for RA were developed to identify RA patients in early disease and focused on features in early arthritis that associated with persistent and/or erosive disease (Table 1) [27]. Radiographic changes were not included as these were not characteristic for early disease. However, it was decided that when erosions typically for RA were present a patient was classified as RA in order to capture also patients with established but inactive disease who did not fulfil the criteria of early disease [27,28]. The new criteria indeed classified more patients in early disease but also resulted in more heterogeneity in patients classified as RA [29]. In line with this, the phenotype at RA presentation and during the course of RA is different when disease is classified according to the 1987 criteria or 2010 criteria [30,31].

Table 1. Classification criteria for RA

1987 ACR criteria [26]	2010 ACR/EULAR criteria [27]
Morning stiffness	Joint involvement*
	1 large joint (0)
Arthritis of 3 or more joint areas	2-10 large joints (1)
	1-3 small joints (2)
Arthritis of hand joints	4-10 small joints (3)
	>10 joints (at least 1 small joint) (5)
Rheumatoid nodules	Serology
	Negative RF and negative ACPA (0)
Serum RF	Low-positive RF or low-positive ACPA (2)
	High-positive RF or high-positive ACPA (3)
Radiographic changes	Acute-phase reactants
	Normal CRP and normal ESR (0)
	Abnormal CRP or abnormal ESR (1)
	Duration of symptoms
	<6 weeks (0)
	≥6 weeks (1)

1987 ACR criteria: patients fulfilling ≥ 4 out of 7 criteria are classified as RA
2010 EULAR/ACR criteria: patients with a score of ≥6 out of 10 are classified as RA
*Refers to any swollen or tender joint on examination

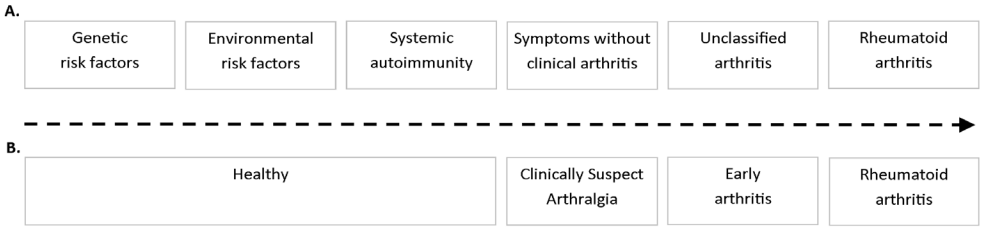
PRECLINICAL RA

RA has a period of preclinical disease. This became well-recognised because of observations that ACPA and RF could be detected several years before the presentation with RA [32,33]. This was studied in cohorts of blood donors from whom multiple blood samples of RA patients were available before their arthritis became clinically detectable. The frequency of autoantibody positivity as well as the level and the epitope spreading increased when approaching the moment of symptom onset [32–35], indicating maturation of the autoantibody response in the preclinical period. Using a similar study approach, markers of systemic inflammation and biomarkers of bone metabolism were found to be increased in the preclinical phase [36–38]. These findings suggested that disease processes within RA can be active years to months before a patient presents with RA.

Recently, the EULAR study group for risk factors for RA formulated terminology to be used during the different preclinical and early phases of RA that could be used as framework for future research [39]. Six phases (phases A-F) of RA development were formulated: (A) genetic risk factors for RA, (B) environmental risk factors for RA, (C) systemic autoimmunity associated with RA, (D) symptoms without clinical arthritis, (E) unclassified arthritis and (F) RA (Figure 1) [39]. It was emphasised that the phases could be used in a combinatorial manner, indicating that a patient can be in two phases concurrently. In addition, patients do not have

to pass through all phases and the phases do not necessarily occur in the same order before RA develops. Importantly, the term 'pre-RA' should only be used retrospectively as it was considered inappropriate to label healthy persons with certain genetic or environmental risk factors as having pre-RA as the majority of them will never develop RA [39]. This latter, is in line with the clinical point of view of development of RA in which a patient is healthy until presenting with complaints (Figure 1).

Figure 1. The phases of RA development as defined by the EULAR study group for risk factors for RA (A) and according to the clinical point of view (B)



CLINICALLY SUSPECT ARTHRALGIA

One of the defined early phases in RA development was the phase of symptoms without clinical arthritis (Figure 1). Although it was widely recognised by the study group that many patients with RA have a period of symptoms that is likely to be related to the development of RA before they develop clinical arthritis, the symptoms that are specific for this early phase were not specified.

Identifying patients in the early symptomatic phase without arthritis is challenging as arthralgia is the main symptom of most patients presenting to rheumatologists and the majority will never develop RA. In addition, to allow studies on this early symptomatic phase it is needed that arthralgia patients with an increased prior chance on RA are identified. This can be done by adding the criterion of having RA-related autoantibodies to the arthralgia [40,41]. Another approach is to make distinctions based on the type of arthralgia. Patients with Clinically Suspect Arthralgia (CSA) have arthralgia that is because of the character of the symptoms considered by their rheumatologist as clinically suspect to progress to RA over time. This approach is based on the clinical expertise of the rheumatologist and proposes that clinical expertise is a valuable tool to select patients with an increased risk of RA. Selecting patients on clinical grounds before ordering additional tests is in line with clinical practice (Figure 1) and allows identifying both autoantibody-positive and autoantibody-negative RA in an early symptomatic phase.

Clinically Suspect Arthralgia cohort

The CSA cohort is an inception cohort that was set up in Leiden in April 2012 to study the early symptomatic RA phase without clinical arthritis. The inclusion criteria are the presence

of arthralgia of the small joints for less than one year which, because of the character of the symptoms, is considered by the rheumatologist as being suspect to progress to RA over time. No further criteria are made with regards to the type of symptoms and thus inclusion is essentially based on the expert opinion of the rheumatologist. Importantly, CSA is not present if clinical arthritis was observed at physical examination or another explanation for the arthralgia was more likely (such as osteoarthritis or fibromyalgia).

At baseline, questionnaires are completed, physical examination performed, blood obtained and X-rays and MRI made. Magnetic Resonance Imaging (MRI) of the MCP2-5, wrist and MTP1-5 joints of the most painful side, or the dominant side in case of equally severe symptoms at both sides, is performed within two weeks after clinical assessment. The joints are scanned with an 1.5 Tesla extremity MRI-scanner using contrast-enhancement and according to the RA MRI scoring system (RAMRIS) protocol [42].

Patients are prospectively followed with scheduled visits at 4, 12 and 24 months. If necessary (for instance when the patient noticed swollen joints) patients are seen in between the scheduled visits by their rheumatologist. Follow-up ends earlier when clinical arthritis has developed.

MRI in Clinically Suspect Arthralgia

Local subclinical inflammation might be present in the early symptomatic phase of RA without clinical arthritis [43–45]. MRI is a very sensitive imaging modality and more sensitive than physical examination to measure local inflammation [42,46]. This makes MRI a suitable tool for evaluating the earliest inflammatory changes in the small joints of patients that might be in the early phase of RA. MRI depicts inflammation of the synovium of joints (synovitis) and tendons (tenosynovitis). In addition, it is the only imaging modality that can depict bone marrow edema (BME), which is also called osteitis in RA and is a strong predictor for progression of joint damage in RA [42,47]. The presence of a validated semi-quantitatively scoring methodology (the Outcome Measures in Rheumatology Clinical Trials (OMERACT) RA MRI scoring system (RAMRIS)) makes MRI very suitable for research as the extent and severity of MRI features can be compared objectively [42,48].

SEVERITY OF RA COURSE

The course of RA is variable between patients; some patients have a disabling, persistent course with severe joint destruction while others have a more mild disease course. The biologic processes underlying these interindividual differences in joint destruction and disease persistence are incompletely understood thus far. In addition, differentiating patients who will develop a severe disease course from patients with a mild disease course is not yet accurate [49,50], hampering individualised treatment.

To evaluate the disease course in RA several outcome measures are used. Disease activity is commonly assessed by the Disease Activity Score (DAS) which is a composite

measure of the swollen and tender joint count, the patient global assessment of the disease activity on a visual analog scale (VAS) and the level of acute phase reactants [51]. Functional disability is mostly measured by the Health Assessment Questionnaire Disability Index (HAQ-DI) which consists of 20 questions in eight categories of functioning [52]. The traditional long-term outcome in RA is the severity of destruction of the small joints, one of the hallmarks of RA [53]. Another important long-term outcome is arthritis persistence.

Joint damage as disease outcome

The severity of damage of the hand and feet joints, assessed on radiographs is the key outcome measure in RA. This outcome measure has several advantages. First, it is considered to reflect the cumulative burden of inflammation and thus represents the disease history [53]. In addition, joint damage is strongly associated with other outcome measures, such as functional disability [55]. And third, it is very suitable as outcome measure for research purposes because of the presence of validated scoring methods that allows evaluating the extent of joint damage on radiographs objectively [53].

The most commonly applied scoring method is the Sharp-van der Heijde scoring (SHS) method. This measure quantitatively evaluates the extent of erosions (range 0-280) and joint space narrowing (which reflects cartilage damage, range 0-168) in both hands and feet [56]. Another scoring methodology is the Larsen score [57], which gives a combined score for erosions and joint space narrowing per joint. The SHS is more sensitive to detect changes over time though also more time-consuming than the Larsen score [53].

To study joint damage, it is preferred that the study population has serial radiographs over time instead of one radiograph at a single time point to capture the progression in joint damage over time adequately. In addition, when investigating specific risk factors for the severity of joint damage the studied patients are ideally untreated to evaluate the risk factor in relation to the natural course of joint damage. The latter may be the case for patients diagnosed and treated in eras when early tailored treatment and use of biologics were uncommon. However, large well-defined longitudinal cohorts including such patients are scarce. To overcome these limitations, results of several (small) cohorts can be combined in meta-analyses and adjustments can be made for the applied treatment strategy.

Risk factors for joint damage progression

Joint destruction is caused by a disbalance between bone degradation and formation. In RA, several inflammatory and immune cell types can be present in the synovial membrane. Two cell types can be considered of particular importance in destruction of bone and cartilage. Synovial fibroblasts are considered important for cartilage degradation. These cells, physiologically involved in the secretion of synovial fluid, can be present abundantly in the synovial membrane in RA and can behave aggressively with invasion of the underlying cartilage. Dysregulated osteoclast activation is mainly involved in bone degradation [1,58]. Why these processes occur and how they are initiated is incompletely understand, but several risk

factors for joint damage have been identified.

The most important risk factor for severe joint damage progression is the presence of autoantibodies, mainly ACPA. Also inflammatory markers are associated with more severe joint damage progression. Autoantibodies and inflammatory markers together explain approximately 30% of variance in joint destruction after 5 years of disease [59].

In addition, genetic factors play a major role in the severity of joint damage as the heritability of the severity of joint damage has been estimated to be 45-58% [60]. Several genetic risk factors for joint damage have been identified thus far and have been replicated in independent cohorts [61], which is needed in the field of genetics to prevent false-positive findings. Most findings were done using candidate gene studies [62–71] that were dedicated to genes associated with RA development or genes involved in inflammation, immunity or bone homeostasis, though hypothesis-free genome-wide association studies (GWAS) have also been used [72]. The *HLA-DBR1* SE was the first identified genetic risk factor for joint damage and similar as for the association of the SE with RA development, SE was not associated with joint damage progression as such but predisposed to ACPA-positive RA that is associated with more severe joint damage [62]. In addition, genetic risk factors were identified in *CD40, C5orf30, IL15, IL2RA, IL4R, DKK1, GRZB, MMP9, OPG* and *SPAG16* [63–72]. However, a large part of the total genetic effect is considered to be still unexplained.

Arthritis persistence as disease outcome

Persistent arthritis is the other hallmark of RA and can be studied by evaluating its opposite: achieving DMARD-free sustained remission which is defined as the sustained absence of clinical arthritis at physical examination without the use of DMARDs (including corticosteroids). This outcome can be considered the most favourable outcome in RA as it approximates cure of RA [54].

Only a few risk factors for arthritis persistence (absence of achieving DMARD-free sustained remission) have been reported and replicated. One of these factors is prolonged symptom duration at treatment start [23,54], which points to the so-called 'window of opportunity' in RA. Another risk factor is the presence of autoantibodies [54,59], but these explain only a proportion of the variance in arthritis persistence as the large majority of autoantibody negative patients have persistent disease and some patients with autoantibodies can achieve remission [73]. The *HLA-DRB1* SE is the only genetic risk factor that has been found associated with arthritis persistence thus far. To get more comprehension into the mechanisms promoting the chronic nature of RA further risk factors for arthritis persistence need to be identified.

AIMS AND OUTLINE OF THIS THESIS

In general this thesis has two main aims:

1. to investigate the early phase with Clinically Suspect Arthralgia
2. to identify genetic risk factors for disease severity in rheumatoid arthritis

The thesis contains three parts.

In **Part I**, the very early phases of RA without clinically detectable arthritis, mainly the phase of Clinically Suspect Arthralgia (CSA), is examined. In **Chapter 2**, it is systematically reviewed what is currently known on the preclinical phases of RA. This was done within the framework of the phases for the preclinical and early phases of RA formulated by the EULAR study group for risk factors for RA. In **Chapter 3**, the CSA approach and the CSA cohort are introduced. The characteristics of patients with CSA are described and the symptoms, signs and serological markers that are related to subclinical inflammation on MRI are studied. **Chapter 4** evaluates whether subclinical MRI-inflammation is, similar as in ACPA-positive arthralgia patients, also present in ACPA-negative arthralgia patients who are considered prone to progress to RA. For comparisons, also healthy controls and ACPA-negative RA patients are evaluated. **Chapter 5** is the first longitudinal study on patients with CSA and investigates progression from CSA to clinically detectable arthritis. Associations between clinical and serological factors and subclinical MRI-inflammation with the development of clinical arthritis are examined. In **Chapter 6**, the diagnostic accuracy of the clinical expertise for CSA is explored. **Chapter 7** describes the process in which a EULAR taskforce develops an expert-opinion based definition for CSA which may serve as the basis for observational studies and trials in this phase.

In **Part II**, genetic risk factors for a more severe course of RA, in particular joint damage progression, are investigated. These studies are mainly performed within the Leiden Early Arthritis Clinic (EAC) cohort [59]. Since 1993 patients with arthritis of at least one joint and symptom duration less than two years have been included in this population-based inception cohort and prospectively followed during yearly visits. **Chapter 8** evaluates the contribution of the known genetic risk factors to the variance in the severity of joint damage progression and to the accuracy of predicting this severity. Chapter 9-11 describes candidate gene studies for the severity of joint damage. In **Chapter 9**, a variant in *FOXO3A* that was reported to associate with joint damage in RA is replicated. In **Chapter 10**, a variant in *SPP1* is studied that was reported to associate with the development of ACPA-negative RA. **Chapter 11** aims to clarify associations of variants in *IL6, IL10, C5-TRAF1* and *FCRL3* that have been reported to associate with joint damage but for which the results of different studies were contradictory. **Chapter 12** focuses on position 11 at HLA-DRB1 which was recently reported to have a strong association with RA development. In **Chapter 13**, genetic risk factors for joint damage are studied in relation to arthritis persistence, another long-term outcome. In **Chapter 14**, serum level osteoprotegerin is studied in relation to arthritis persistence.

In **Part III**, other outcomes are studied. In **Chapter 15**, it is investigated whether the occurrence of DMARD-free sustained remission is promoted by improved treatment strategies and the relevance of achieving this outcome from patient perspective. **Chapter 16** focuses on fatigue in RA; its eight year course and associations with inflammation and improved treatment strategies are studied.

Finally, **Chapter 17** provides a summary and discussion of the results that are described in this thesis.

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Part I

Phase of Clinically Suspect Arthralgia

Review: The preclinical phase of rheumatoid arthritis. *What is acknowledged and what needs to be assessed?*

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2

Introduction

Rheumatoid arthritis (RA) affects 1% of the population worldwide and is characterised by persistent inflammation and joint damage. It has repeatedly been observed that early initiation of disease-modifying therapy reduces the severity of the disease course, as measured by fewer signs and symptoms and less structural damage. Early treatment is associated with less severe joint damage progression and increased chances of achieving disease-modifying antirheumatic drug-free sustained remission [1,2]. Observations that treatment in the very early phase of RA is more effective, conceivably because the load of disease cells is smaller or because disease mechanisms are not yet settled, have led to increased interest in the earliest disease phases. Ideally, this period is used to modify the disease course and improve the outcome of RA. The timeframe of this treatment-susceptible period is, however, unknown. Several recent studies have provided data indicating that disease processes are already active in the preclinical phase (the period before arthritis becomes clinically detectable). Consequently, it is at present unclear when RA actually starts. The concept that the disease starts when arthritis has become (clinically) detectable is no longer valid.

Herein we systematically review what is presently known of the preclinical phase of RA. With the assistance of a medical librarian, we performed a search for the central terms "rheumatoid arthritis," "preclinical," "autoantibody-positive arthralgia," and "developing RA" in the medical literature databases Medline (Ovid version), PubMed, EMBase (Ovid version), and Web of Science up to December 2012 (Figure 1). Findings from the identified articles, combined with additional hand-searched articles from the reference lists of the identified articles, are summarized here. This overview will lead to the identification of research items that need to be explored in order to identify patients in the preclinical phase who will develop RA. This may ultimately allow individualized interventions during the preclinical phase.

Basis of pre-RA

Although interest in the preclinical phase has increased considerably during the last few years, the idea that disease processes related to RA occur before arthritis is clinically detectable was proposed more than two decades ago. The prevalence of rheumatoid factor (RF) in the preclinical phase of RA was first observed in Finnish and Icelandic patients with RA [3,4]. Increased prevalences of antifilaggrin and antiperinuclear antibodies were also reported [5,6]. The first large longitudinal population study of pre-RA was performed in the high-risk population of Pima Indians, showing that the presence of RF was a risk factor for the development of RA, and that this risk increased in parallel with the RF level [7]. Similar observations were noted in a longitudinal study that evaluated another high-risk population, multicase families [8,9]. These studies have formed the basis for what is now called pre-RA.

Approximately 15 years later, the pre-RA phase received renewed attention. Rantapää-Dahlqvist et al [10] and Nielen et al [11] studied serum samples from RA patients collected serially

in the preclinical phase and observed that the prevalence of autoantibodies increased over time and that this increase can take place even years before RA becomes clinically evident. These two studies served as subsequent landmark studies, after which the number of publications on systemic and local responses and symptoms in the preclinical phase of RA rapidly increased (Figure 1).

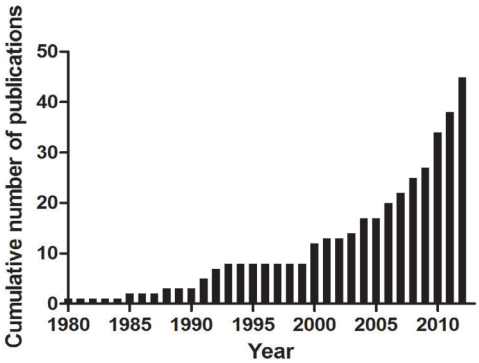

Figure 1. Number of original publications on the preclinical phases of RA. In total, 964 references were extracted from the medical databases Medline (Ovid version), PubMed, EM-Base (Ovid version), and Web of Science. Animal studies, reviews, conference abstracts, case reports, case series, studies including patients 18 years of age, and studies in languages other than English were excluded. A total of 66 unique publications on systemic autoimmunity (phase C) and other systemic or local responses associated with RA and symptoms without clinical arthritis (phase D) published before December 1, 2012 were identified.

Definition of pre-RA

Many different terms are used to describe the phases that occur before clinically manifest RA. These include "pre-RA," "preclinical RA," and "(very) early RA." In order to achieve homogeneity in terminology, in 2011 the study group for risk factors for RA, established by the European League Against Rheumatism (EULAR) Standing Committee on Investigative Rheumatology, formulated a recommendation for terminology to be used with regard to the preclinical and earliest clinically apparent phases of RA [12]. Six phases (phases A-F) of RA development were formulated. These are phase A, genetic risk factors for RA; phase B, environmental risk factors for RA; phase C, systemic autoimmunity associated with RA; phase D, symptoms without clinical arthritis; phase E, unclassified arthritis; and phase F, RA (Figure 2A). It was emphasized that patients do not have to pass through all phases and that the phases do not necessarily occur in the same order before RA eventually develops. In addition, a patient can be in two phases concurrently. Importantly, it was also recommended that the term "pre-RA" only be used retrospectively. This recommendation was made since if all persons who carry certain genetic risk factors or who are exposed to certain environmental risk factors were labeled as having pre-RA, many persons who will never develop RA would inappropriately have been classified as being in a predisease stage. The proposed phases are relevant since they form a framework for future research. Below we review the data available on preclinical arthritis within the framework of these study group formulated phases.

Genetic risk factors for RA

The first phase at which individuals develop an increased risk of RA is at conception, when a subject inherits risk alleles for RA from his or her parents (phase A). More than 40 such risk alleles are currently known, and the majority of these variants are commonly present. When evaluating the frequencies of these risk alleles in the population, the chance that an individual carries none of the RA risk alleles is 7.1×10^{-13}%; in other words, almost everyone carries one or several of these risk alleles. This calculation underlines the relevance of using the term "pre-RA" only retrospectively. These genetic variants have small effect sizes, and a large proportion of the population carrying risk alleles never develops RA.

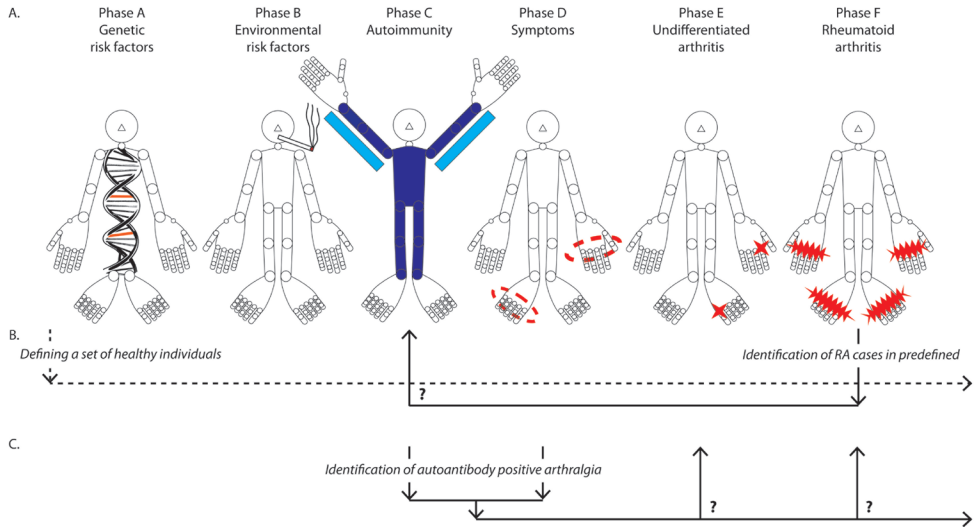

Figure 2. Overview of the preclinical phases of RA and the designs of the studies of the preclinical phases that have been performed. (A) The six phases of preclinical and earliest clinically apparent RA, as defined by the EULAR study group for risk factors for RA. (B) Nested case-control study design. A predefined set of subjects (e.g., blood donors) is followed up. From this set of subjects, RA cases are identified and for each case, a specified number of matched controls who have not developed RA is selected. Biomarkers are compared between preclinically collected samples from these cases and the controls. (C) Prospective cohort study design. Autoantibody-positive arthralgia patients are identified and followed up prospectively.

Environmental risk factors for RA

The heritability of RA has been estimated at 60% [13], implying that 40% of the variance in developing RA might be explained by environmental risk factors (phase B). Many environmental risk factors have been studied, and smoking is the best replicated environmental risk factor [14,15]. Smoking predisposes to RA particularly in patients who carry specific HLA-DRB1 alleles, e.g., smokers carrying two HLA-DRB1 alleles have a 21-fold increased risk of developing anti-citrullinated peptide antibody (ACPA)-positive RA [16]. Despite the high odds ratio in this subgroup, a large majority of smokers do not develop RA. Weaker interactions have also been demonstrated between other genes and smoking [17].

The genetic and environmental risk factors conceptually constitute the earliest preclinical phases of RA. These risk factors have already been known for some time, and an extensive discussion of these risk factors is beyond the scope of this review.

The next two preclinical phases, "developing systemic autoimmunity associated with RA" (phase C) and "symptoms without clinical arthritis" (phase D), have been studied in the last few years. This has been done using mainly two different study designs: nested case-control studies and prospective cohort studies. As will be discussed, the design of the study determined the sort of outcome that was obtained and the conclusions that can be drawn.

From RA back to pre-RA systemic autoimmunity

Studies associating RA with systemic autoimmune responses in the preclinical phase (phase C, with or without phase A and B) were mainly performed using a nested case-control study design (also called case-control studies in a cohort) (Figure 2B). In this type of study, RA cases were identified who were members of a predefined dataset, e.g., a cohort of blood donors from whom blood samples were obtained at least once [10,18]. For each RA patient, a specified number of matched controls who had not developed RA was selected from the same dataset. Consequently, blood samples from RA cases that were collected and stored years before the onset of arthritis could be compared with blood samples from matched controls.

Compared to full prospective cohort studies, the main advantage of this study design is the smaller number of study subjects that is required, which coincides with lower efforts and costs. However, this study design also has limitations. The cases and controls are selected, which may give rise to sampling error and bias. Second, the time of onset of symptoms and arthritis in the cases is not exactly known, leaving the timing of the appearance of autoantibodies in relationship to the onset of symptoms unexplored. Furthermore, in several studies the healthy controls were not carefully evaluated for rheumatic diseases; this might have led to an overestimation of the prevalence of autoantibodies in controls and an underestimation of the specificity. Using nested case-control studies, the biomarkers described below have been identified to be abnormally regulated in the preclinical phases of RA (see also Table 1).

Autoantibodies - In the nested case-control studies by Rantapää-Dahlqvist et al [10] and Nielen et al [11], 83 Swedish patients with RA (73.1% IgM-RF-positive and 70.1% ACPA-positive) and 79 Dutch patients with RA (frequencies of autoantibodies at diagnosis not reported), respectively, who were donors to a blood bank before symptom onset were studied [10,11]. A total of 98 pre-RA blood samples were available for the patients in the Swedish study, and a total of 1,078 pre-RA blood samples were available for the patients in the Dutch study. In the Swedish study, the presence of IgM-RF and ACPA was reported in 19.3% and 33.7%, respectively, of the RA cases within 10 years before RA diagnosis, compared to 6.0% and 1.8%, respectively, of the matched controls [11]. Similarly, in the Dutch dataset, 27.8% of the patients

Table 1. Results of the nested case-control studies of abnormalities in the preclinical phase of RA*

Author, year	Cohort	No. of cases (total no. of samples)	Time between (first) sample and RA	Measured factors	Main result
Autoantibodies					
Rantapää-Dahlqvist et al, 2003 [10]	Northern Sweden health and disease study and Maternity cohort of Northern Sweden	83 (98)	Median 2.5 years	IgG-RF, IgM-RF, IgA-RF, ACPA; sensitivity, specificity, PPV and NPV of autoantibodies	Increased prevalence of all autoantibodies (IgM-RF 19%, ACPA 34%) in pre-RA. Sensitivity: ACPA 34%, IgM-RF 20% Specificity: ACPA 98%, IgM-RF 95% PPV†: ACPA 16%, IgM-RF 4% NPV†: ACPA 99%, IgM-RF 99%
Nielen et al, 2004 [11]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	IgM-RF and ACPA; sensitivity, specificity, PPV and NPV of autoantibodies	Increased prevalence of IgM-RF (28%) and ACPA (41%) in pre-RA. Sensitivity: ACPA 29%, IgM-RF 21% Specificity: ACPA 99%, IgM-RF 100% PPV‡: ACPA 5%, IgM-RF 2%
Majka et al, 2008 [22]	Department of defense serum repository, US	83 (243)	Mean 6.6 years	IgM-RF and ACPA	Increased prevalence of IgM-RF (57%) and ACPA (61%) in pre-RA. Period of time that autoantibodies are present before diagnosis lengthens as the age at time of diagnosis increased.
Chibnik et al, 2009 [23]	Nurses' Health Study	93 (93)	Mean 5.6 years	ACPA level; sensitivity, specificity and hazard for various ACPA thresholds.	Higher ACPA levels were associated with shorter time to RA diagnosis; lower threshold for ACPA positivity more sensitive in predicting RA development.
Van der Woude et al, 2010 [25]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	36 (36)§	Median 2.5 years	Recognition of 5 citrullinated peptides	Number of recognized peptides increased in the pre-RA period.
Kolfenbach et al, 2010 [62]	Department of defense serum repository, US	83 (243)	Mean 6.6 years	Anti-PAD-4	Anti-PAD-4 prevalence of 18.1% in pre-RA and its presence was associated with ACPA-positivity.

Van de Stadt et al, 2011 [26]	Sanquin blood bank, the Netherlands	53 (374) §	Median 1.0 years	Recognition of 5 citrullinated peptides	Number of recognized peptides increased over time in the pre-RA phase without a dominant epitope spreading pattern.
Autoantibodies & markers of systemic inflammation					
Jørgensen et al, 2008 [18]	Blood bank, Norway	49 (49)	Median 9.3 years	IgM-RF, ACPA, 16 cytokines and related markers	Increased prevalence of IgM-RF (20%) and ACPA (31%) in pre-RA; increased prevalence of raised TNFα levels within 5 years pre-RA.
Kokkonen et al, 2011 [33]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	71 (71)	Median 2.5 years	Isotypes of ACPA (IgG, IgM, IgA) and 29 cytokines and chemokines	Increased prevalence of mainly IgG-ACPA and IgA-ACPA in pre-RA; different pattern of up-regulated chemokines in IgG-ACPA and IgA-ACPA-positive pre-RA.
Sokolove et al, 2012 [24]	Department of defense serum repository, US	81 (±283)	Mean 6.4 years	ACPA reactivity 48 cytokines	Gradual increase in number of ACPA subtypes is followed by a parallel increase in cytokines in pre-RA approaching symptom onset.
Autoantibodies & markers of bone metabolism					
Turesson et al, 2011 [21]	Malmö Diet and Cancer Study, Sweden	169 (169)	Median 5 years	IgM-RF, ACPA, anti-MCV and COMP	Increased prevalence of IgM-RF (19%), ACPA (22%) and anti-MCV; no increased COMP level in preclinical phase of RA cases; increased prevalence of elevated COMP level within 3 years prior ACPA-negative RA.
Markers of systemic inflammation					
Aho et al, 2000 [28]	Community cohort, Finland	124 (124)	Upper limit 20 years	CRP	No increased CRP levels in pre-RA.
Masi et al, 2001 [27]	Community cohort, US	18 (18)	Median 12 years	CRP and acute SAA	Increased CRP levels in men with pre-RA .
Nielen et al, 2004 [32]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	CRP	Increased CRP levels, mainly within 2 years prior symptom onset in pre-RA.
Shadick et al, 2006 [20]	Women's health study, US	90 (90)	Mean 6.6 years	CRP	No association between increased CRP levels in pre-RA and development of RA
Nielen et al, 2006 [34]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	sPLA2, CRP, IgM-RF and ACPA	No time lag between development of acute phase reactants and autoantibodies in pre-RA.

Rantapää-Dahlqvist et al, 2007 [29]	Northern Sweden Health and Disease Study and Northern Sweden Maternity cohort	92 (92)	Median 3.3 years	sPLA2, CRP, IL-6 and MCP-1	Increased MCP-1 level in IgM-RF or ACPA-positive pre-RA.
Karlson et al, 2009 [30]	Nurses' health study and Women's health study, US	170 (170)	Mean 5.2 years	CRP, IL-6 and sTNFRII	Association between sTNFRII level in pre-RA and development of RA.
Kokkonen et al, 2010 [19]	Medical biobank Northern Sweden	85 (85)	Median 3.3 years	29 cytokines and related factors and chemokines	Increased levels of 18 of the analytes in pre-RA, particularly in ACPA or IgM-RF-positive RA.
Deane et al, 2010 [31]	Department of Defense Serum Repository, US	73 (212) ¶	Mean 6.6 years	CRP and 14 cytokines and chemokines	Increased levels of CRP, cytokines and chemokines in pre-RA. Predicted time to diagnosis based on number of elevated cytokines/chemokines increased with age.
Biomarkers of bone metabolism					
Schaardenburg et al, 2011 [35]	Sanquin blood bank, the Netherlands	79 (191)	1, 2 and 5 years	Osteocalcin, P1NP, β-CTX, osteoprotegerin and RANKL	Increased levels of P1NP and osteoprotegerin and no increased levels of osteocalcin, β - CTX and RANKL in pre-RA.
Lipid profile and cardiovascular disease					
Maradit-Kremers et al, 2005 [38] #	Population-based incidence cohort, Minnesota, US	603	NA	Hospitalized MI, unrecognized MI, coronary revascularization procedures, angina pectoris, out-of-hospital sudden death	Increased number of hospitalized and unrecognized MIs and decreased number of angina pectoris cases in pre-RA.
Van Halm et al, 2007 [36]	Sanquin blood bank, the Netherlands	79 (1,078)	Median 7.5 years	Total cholesterol, HDL, triglycerides, Apo A-I, Apo B, Lp(a)	Increased levels of total cholesterol, triglycerides and Apo B and decreased level of Apo A-I in pre-RA.

Holmqvist et al, 2009 [39]	National Early Arthritis Register and Epidemiologic Investigation of RA case-control study of incident RA, Sweden	10,579	NA	CHD, MI and angina pectoris	No increased occurrence of CHD, MI or angina pectoris in pre-RA.
Myasoedova et al, 2010 [37]	Population-based inception cohort Minnesota, US	577 (3,048)	Within 5 years pre-RA to 5 years post-RA	Total cholesterol, HDL, LDL and triglycerides	Lower prevalence of increased total cholesterol or LDL in pre-RA; significant decline in total cholesterol and LDL levels in the 5 years pre-RA.
Kerola et al, 2012 [40] #	Based on a nationwide register on special reimbursements for medication costs, Finland	7,209	NA	CHD and chronic hypertension	Slightly increased prevalence of CHD and no increased prevalence of chronic hypertension at the time of RA diagnosis.

*All results are based on comparing cases with pre-RA to matched non-RA controls. †Positive predictive value (PPV) and negative predictive value (NPV) for, respectively, developing and not developing RA at any point in life; the population-based pretest frequency of RA was set as a pretest probability. ‡PPV for developing RA within 5 years; the population-based pretest frequency of RA was set as a pretest probability. §All ACPA-positive. ¶ All IgM-RF and/or ACPA-positive. #These studies are not defined as nested case-control studies, but as retrospective cohort studies in which the prevalence of cardiovascular disease in the pre-RA period was retrospectively examined in RA patients and compared to matched controls in the same population. Anti-PAD-4=anti-peptidylarginine deiminase 4; anti-MCV=anti-mutated and citrullinated vimentin; COMP=cartilage oligomeric matrix protein; SAA=serum amyloid A; sPLA2=secretory phospholipase A2; MCP-1=monocyte chemotactic protein 1; sTNFRII=soluble tumor necrosis factor receptor type II; PINP=N-terminal type I procollagen propeptide; β-CTX=C-terminal crosslinking telopeptide of type I collagen; MI myocardial infarction; Apo A-I apolipoprotein A-I; Lp(a) lipoprotein(a); CHD coronary heart disease.

were IgM-RF-positive and 40.5% were ACPA-positive within 15 years prior to RA diagnosis, compared to 1.1% and 0.6%, respectively, of the matched controls [10]. Similar frequencies of IgM-RF and ACPA in the pre-RA phase were reported in several cohorts in Norway, Sweden, and the US [18–21]. Even higher frequencies were reported in the pre-RA phase in a military cohort in the US (57% IgM-RF-positive and 61% ACPA-positive) [22]. Comparison of these frequencies with the prevalence of IgM-RF and ACPA observed in other patients with early arthritis (58% and 53%, respectively [2]) is potentially scientifically incorrect; nonetheless, this comparison suggests that in a large proportion of autoantibody-positive RA patients the autoantibodies are already present in the preclinical period.

Based on the proportion of RA cases with autoantibodies in the preclinical phase (sensitivity) and the proportion of controls without autoantibodies (specificity) in the studied cohorts, the risk of developing arthritis for individuals with autoantibodies in the general population was estimated. In these calculations the population-based frequency of RA was set as a pretest probability. The risk of developing RA within 5 years [11] and at any time in life [10] was estimated at 1.5% and 4%, respectively, in subjects who were IgM-RF-positive and 5.3% and 16%, respectively, in subjects who were ACPA-positive.

Regarding the time course of autoantibody development, the frequency of autoantibody positivity as well as the levels of these autoantibodies increased approaching the onset of symptoms [10,11,18,22,23]. It is unclear whether IgM-RF or ACPA appears earlier in time. Although in the Swedish cohort IgM-RF could be detected earlier than ACPA [10], in the Dutch cohort ACPA was detected earlier than IgM-RF (median duration from seroconversion to symptom onset 2.0 years for IgM-RF and 4.8 years for ACPA) [11]. In a US cohort, IgM-RF and ACPA appeared concurrently in the pre-RA phase (6.0 years for IgM-RF and 5.4 years for ACPA) [22]. With regard to the ACPA response, the number of epitopes to which the response is directed increased over time and in parallel with the increase in ACPA level approaching the onset of symptoms, revealing that this response matures in the preclinical disease phases [24–26].

Markers of systemic inflammation - Various acute-phase reactants, cytokines, cytokine-related factors, and chemokines were measured in serum samples that were collected once (one sample per case) or serially from RA patients prior to symptom onset. In the studies in which serum was obtained at a single time point, the period between sample collection and RA diagnosis was variable, with a maximum of 20 years; only a small number of samples were obtained within one year of symptom onset. These studies with single samples do not allow drawing conclusions with regard to the evolution of systemic markers of inflammation over time before arthritis onset, and negative findings are difficult to interpret since they might potentially be the result of analyzing samples that were obtained too early.

None of the single serum sample-based studies, except for one [27], demonstrated increased levels of C-reactive protein (CRP) or other acute-phase reactants (i.e., secretory

phospholipase A2) during the pre-RA phase, irrespective of autoantibody status or time to diagnosis [20,27–30]. However, a study evaluating serially collected serum samples from autoantibody-positive RA cases demonstrated increased levels in significantly more cases than controls [31]. Other investigators measured CRP serially over time in serum samples collected from RA patients during the preclinical period and observed a statistically significant increase in median CRP levels, although within the normal range, in the periods 4-5 years, 1-2 years, and 0-1 years prior to diagnosis [32]. A gradual increase in CRP level was observed both in autoantibody-positive and autoantibody-negative cases, with the highest level observed at the time closest to arthritis onset. Furthermore, at all preclinical time points studied, the autoantibody-positive patients had slightly higher CRP levels than the autoantibody-negative patients [32].

Over 30 different cytokines have been studied using different techniques with different sensitivities; the majority of these were measured in the single serum sample-based studies. Since the time period between collection of serum sample and RA onset was variable in these studies, the results cannot be easily compared to look for replication. Nonetheless, the levels of some cytokines (tumor necrosis factor α [TNFα] and/or the soluble TNF receptor type I/II, which parallels TNFα levels, and interleukin-6 [IL-6]) were increased during the preclinical phase in several studies. Both TNFα and IL-6 levels were significantly increased during the pre-RA phase in most [18,19,24,30,31,33] but not all studies [18,29,33]. More variable results were found for other markers, including different interleukins, granulocyte-macrophage colony-stimulating factor, monocyte chemotactic protein 1, and interferon-γ. In the presence of increased cytokine levels, these results were most often found in both autoantibody-positive and autoantibody-negative cases, but autoantibody-positive cases had generally higher levels than the autoantibody-negative cases [19,24,29]. These levels were also the highest close to the diagnosis of RA [19].

It remains unclear whether levels of the inflammatory markers increase before, after, or simultaneously with the development of autoantibodies. No time lag was found between the increase in CRP level and the presence of autoantibodies in some studies [32,34], whereas another study suggested a longer predating period for autoantibodies than for increased cytokine levels [18]. Consistent with this finding, a recent study showed that the increase in ACPA level was followed by an elevation in cytokine levels [24]. Interestingly, Deane et al studied serum samples from cases that were ACPA-negative but would later in the pre-RA period become ACPA-positive and showed higher proportions of positive cytokines in these samples than in control samples, suggesting that the increase in autoantibodies occurred later in time than the change in the levels of inflammatory markers [31].

Biomarkers of bone metabolism - Two nested case-control studies examined some markers of bone metabolism during the preclinical phase, but no definitive conclusions could be drawn. One study reported a higher prevalence of increased levels of cartilage oligomeric

Table 2: Results of the longitudinal cohort studies in autoantibody-positive arthralgia*

Author, year	Cohort†	No. of cases	Progression to arthritis (% of subjects)	Median duration from study entry to diagnosis of arthritis, months	Median duration of follow-up, months	Measured factors	Main result
Autoantibodies							
Bos et al, 2010 [41]	Amsterdam, the Netherlands (cohort 1)	147	20	11	28	IgM-RF and ACPA levels, CRP and shared epitope	The presence of ACPA, but not IgM-RF was associated with progression to arthritis.
Autoantibodies & markers of systemic inflammation							
Van de Stadt et al, 2011 [45]	Amsterdam, the Netherlands (cohort 1)	244	28	11	36	IgM-RF and ACPA levels, reactivity to 5 citrullinated peptides and shared epitope	Broader ACPA repertoire in case of development to arthritis. Similar CRP levels in patients with and without arthritis development.
Markers of systemic inflammation							
Van Baarsen et al, 2010 [42]	Amsterdam, the Netherlands (cohort 1)	109	18	7	NP	Gene expression profile	Signatures associated with arthritis development were involved in IFN-mediated immunity, hematopoieses and chemokine/cytokine activity.
Limper et al, 2012 [47]	Amsterdam, the Netherlands (cohort 1)	137	26	11	21	CRP, PCT, sPLA2, TNFα, IL-6, IL-12p70, IL-10, IFN-γ and 21 mRNA biomarkers	Similar biomarker levels in patients with and without progression to arthritis during follow-up.
Lipid profile & cardiovascular risk							
Van de Stadt et al, 2012 [48]	Amsterdam, the Netherlands (cohort 1)	348	33	12	24	Total cholesterol, HDL, LDL, triglycerides, Apo A-I and Apo B	After adjusting for ACPA status, only an association was seen between decreased level of Apo A-I and progression to arthritis.

De Hair et al, 2012 [49]	Amsterdam, the Netherlands (cohort 2)	55‡	27	13	27	BMI ≥25 kg/m^2 and smoking	Association between overweight on smoking on progression from arthralgia to arthritis.
Local inflammation on imaging							
Van de Stadt et al, 2010 [44]	Amsterdam, the Netherlands (cohort 1)	192	23	11	26	Joint effusion, arthritis, tenosynovitis and power Doppler signal on ultrasonography of tender and contralateral joints	Significant association at joint level between ultrasonography abnormalities and progression from arthralgia to arthritis, only a positive trend was seen at the level of the patient.
Van de Sande et al, 2011 [43]	Amsterdam, the Netherlands (cohort 2)	13	31	3	37	Dynamic contrast-enhanced MRI and synovial biopsy of knee	No differences on MRI and immuno-histochemical findings synovial biopsy in cases with and without progression to arthritis and controls.
Gent et al, 2012 [46]	Amsterdam, the Netherlands (cohort 1)	29§	31	NP	24	Macrophage PET MCPs, PIPs and wrists	All of the 4 cases with increased uptake on PET and another 5 cases without increased uptake progressed from arthralgia to arthritis.
Combining risk factors							
Van de Stadt et al, 2013 [63]	Amsterdam, the Netherlands (cohort 1)	374	35	12	32	Developing of a prediction model	The AUC value of a model with 9 variables was 0.82 (95% CI 0.75-0.89).
Preventive trials							
Bos et al, 2010 [57]	Amsterdam, the Netherlands (cohort 1)	83	20-21	NP	26	Two intramuscular injections with dexamethasone or placebo at baseline and 6 weeks	No differences in arthritis development; lower levels of ACPA and IgM-RF up to 6 months.

* All results are based on a single time point value at the time of inclusion in the cohort. †Two different cohorts (cohort 1 and cohort 2) from Amsterdam were examined in these studies and included IgM-RF and/or ACPA-positive individuals with nontraumatic arthralgia, unless indicated otherwise. ‡IgM-RF and/or ACPA-positive individuals with arthralgia (n=51) or without arthralgia with a family history of RA (n=4). §All ACPA positive. NP=not provided.

matrix protein, but this was only present in a subgroup of autoantibody-negative patients during the period closest to diagnosis [21]. The other study reported an increase in N-terminal type I procollagen propeptide and osteoprotegerin; this latter finding is not straightforward to explain [35].

Lipid profile and cardiovascular disease - Whether the lipid profile is changed in the preclinical phase is also unknown. Although a more atherogenic lipid profile was found in preclinical serum samples from RA patients compared to controls in one study [36], in a North American study increased levels of proatherogenic factors were less prevalent in pre-RA cases compared to controls [37]. Large studies were performed on the risk of cardiovascular disease, and contradictory results were also observed in those studies. Both increased and similar prevalences of myocardial infarction and coronary heart disease in patients in the preclinical phase of RA compared to controls were reported [38–40].

From autoimmunity with symptoms to clinical arthritis

In the nested case-control studies described above, the phases F (RA) and C (systemic autoimmunity) were studied, without addressing the phase in between (phase D, symptoms without clinical arthritis). A combination of phase C (systemic autoimmunity, with or without phase A and B) and phase D, however, was the starting point of prospective studies that examined progression from autoantibody-positive arthralgia to the onset of arthritis. The large majority of the published prospective data originate from the same cohort of patients with arthralgia in Amsterdam, the Netherlands. Patients with a combination of any kind of nontraumatic arthralgia and IgM-RF or ACPA positivity were recruited and followed up [41]. After a median duration of 7–12 months, 18–35% of the autoantibody-positive arthralgia patients developed clinically detectable arthritis [41–48]. Interestingly, as described above, in patients without symptoms (phase C) the chance of RA development in those who were ACPA-positive was estimated to be 5.3% [11] and 16% [10] and thus was seemingly lower than the chance observed in patients with autoantibodies and arthralgia included in the Amsterdam study. In this cohort study there were no requirements regarding the type of symptoms, which is consistent with the description of phase D by the EULAR study group [12] where the type of symptoms that predispose to RA were also not explicated. Intriguingly, however, in a subanalysis of patients with so-called inflammatory arthralgia (defined as symmetric arthralgia of small joints), 6 of 10 patients developed arthritis [41]. The risk factors for progression to clinical arthritis that were identified are described below (see also Table 2). For most factors, identification was based on one cohort study and replication in other longitudinal studies is still lacking.

Autoantibodies - The presence and level of ACPA [41] and the number of recognized epitopes [45] were associated with arthritis development (90% of the arthralgia patients who developed arthritis were ACPA-positive, compared to 58% of the patients who did not develop

arthritis). In this cohort, the presence of IgM-RF (but not its level) was only associated with arthritis development in the concomitant presence of ACPA [41].

Markers of systemic inflammation - Although some markers showed a trend toward higher levels in the patients who developed arthritis, no acute-phase reactant or cytokine was significantly associated with an increased risk of arthritis [41,47].

Lipid profile and cardiovascular risk - Slight differences were seen in lipid profile between the patients whose symptoms did and those whose symptoms did not progress to arthritis. After adjustment for ACPA status, a lower apolipoprotein A-I level was associated with progression to arthritis [48]. Another observational study of the Amsterdam cohort examining IgM-RFM- and/or ACPA-positive individuals with arthralgia or individuals with a family history of RA showed that smoking and being overweight were associated with arthritis development [49].

Imaging of local joint inflammation in the preclinical phase - Thus far, we have not yet discussed whether there is local inflammation in the joints in the preclinical phase. Practical, and perhaps also ethical, hurdles hamper performing synovial biopsies in patients with symptoms without clinical arthritis. Nonetheless, inflammation was demonstrated in synovial tissue from clinically uninvolved knees in RA patients who later developed clinically detectable arthritis in the biopsied knee, indicating that a phase of asymptomatic arthritis precedes clinical arthritis [50].

Recently, three different imaging modalities (ultrasonography, magnetic resonance imaging [MRI], and positron emission tomography [PET]) were used in cross-sectional or prospective studies of autoantibody-positive patients with arthralgia, assessing the presence of local subclinical inflammation. The study that used ultrasonography demonstrated abnormalities that were associated with progression to arthritis at the level of the joint but not at the level of the patient [44]. More studies of ultrasonography are required to evaluate whether it is valuable for identifying preclinical inflammation.

Another study from Amsterdam used PET and observed increased uptake on the hand or wrist in 4 of the 9 ACPA-positive arthralgia patients who later developed arthritis [46]. A comparable study evaluating MRI of the knees of autoantibody-positive arthralgia patients did not find differences between patients who did and those who did not progress to clinical arthritis. It was not reported what proportion of the scanned patients had symptoms in the scanned knee [43]. A recent cross-sectional MRI study was performed on a different set of ACPA-positive arthralgia patients than those included in the prospective cohort described above and evaluated inflammation using MRI of the hand and foot joints [51]. ACPA-positive patients with arthralgia of small joints had higher MRI inflammation scores than healthy controls, but lower scores than ACPA-positive RA patients. Furthermore, the MRI inflammation levels were significantly associated with the CRP levels in the ACPA-positive

arthralgia patients [51]. These data support the notion that there is also local inflammation in the preclinical phase of RA. Despite these positive initial findings, the numbers of patients included in these studies were small, and further imaging studies are needed to increase insight into processes occurring locally in the joint in the preclinical phase.

Summary of what is acknowledged

In summary, multiple studies have evaluated various biomarkers in the preclinical phases of RA. There is convincing evidence that autoantibody development and maturation occurs before clinically detectable arthritis develops. The time course between autoantibody and symptom development is still indefinite, and multiple large prospective studies starting in the symptom-free period have not been published. Furthermore, based on the data available, there is suggestive evidence that inflammation occurs in the preclinical phase of RA, both locally in the joint and measurable in the systemic circulation. Some of the results on this subject are contradictory, however, as increased levels of acute-phase reactants and cytokines were observed in some of the nested case-control studies, but not in the prospective cohort study. The time course between the appearance of autoantibodies and inflammation also remains to be elucidated.

There are factors that should be taken into consideration when interpreting the results of the studies reviewed here. The number of RA cases in most studies was not large, and in the prospective studies depended on the duration of follow-up. In addition, the available findings obtained in prospectively followed up autoantibody-positive arthralgia patients are largely based on a single Dutch cohort. Hence, these data are not yet replicated and the generalizability of the findings has not yet been assessed. Future studies in large and different cohorts are required for validation.

What subsequently needs to be assessed?

Although evidence has emerged that autoimmune deregulation starts in the preclinical phase, several major issues are yet unexplored. Table 3 provides a research agenda. The biologic basis for the development of RA-related autoimmunity is not yet elucidated. It has been suggested that RA-related autoantibodies are generated on extraarticular sites and are associated with mucosal inflammation [52–54]. Associations with periodontitis, antibodies to Porphyromonas gingivalis [52,53], and airway abnormalities [54] have been described, but the causality is unclear.

Observations that autoantibodies against citrullinated vimentin can activate osteoclasts [55] and that the presence of RF is a significant predictor of cardiovascular events and mortality in individuals without RA [56] suggest that the presence of these autoantibodies is harmful. Given the prevalence of ACPA in the general population of 1%, a relevant question is to consider when the presence of autoantibodies is benign or detrimental.

For individuals with abnormal test results, e.g., the presence of RA-related

autoantibodies or abnormalities on imaging, the main question is what the absolute risk is for progression to arthritis. These risks may differ in the presence of several other abnormal test results and also depend on the presence or absence of certain symptoms. Furthermore, in order to explore the preclinical phase at the individual level, the persons who will progress to developing arthritis and RA need to be identified with high accuracy (for instance with a greater than 80% chance of arthritis/RA development if test results are positive). The development of such risk stratification is basic to the design of preventive trials. To date, one preventive trial has been performed in a total population of autoantibody-positive arthralgia patients; dexamethasone was not effective in the median study period of 26 months [57].

Longitudinal cohort studies

Large and multiple prospective follow-up studies are needed to answer these questions and to validate the answers. Fortunately, several initiatives have been formed to establish such cohorts, starting with either the general population or high-risk populations (e.g., family members of RA patients, arthralgia patients, or ACPA-positive individuals). One of the approaches of the Studies of the Etiology of RA (SERA) group in the US and of El-Gabalawy et al in studying North American Native populations in Canada is to start with persons who are at increased risk because of family history [58]. These first-degree relatives of RA patients who do not themselves have clinical arthritis are being followed up. The Canadian North American Native population forms a unique population to identify individuals who may be in the pre-RA period because of the high prevalence of ACPA (4-19%) and RA (±3%) [59]. Cross-sectional baseline results of these studies have already shown higher levels of multiple inflammation markers [60] and an association of some inflammation markers with autoantibodies in these first-degree relatives without clinical arthritis [61].

The two available cohorts of autoantibody-positive individuals from Amsterdam, the Netherlands, are extensively described in this review [41,43]. The Leiden approach is to longitudinally study patients with "clinically suspect arthralgia." This indicates arthralgia that, because of the character of the symptoms, the rheumatologist suspects will progress to arthritis over time; IgM-RF or ACPA-positivity is not required. The presence of local inflammation in all of these persons will be studied in detail with dedicated extremity MRI of the wrist, hand, and foot joints.

Hopefully, the results of future studies will increase our understanding of the processes occurring during the preclinical phase of RA and enable targeted intervention in these processes before the clinical picture that is characteristic for RA has evolved.

Table 3. Research agenda for examining the preclinical phase of RA

1. Where (in what tissue or organ) does inflammation in the light of RA start?
2. What is the timing between genetic factors, environmental exposures, and the development of autoimmunity and arthritis? Similarly, what is the temporal relationship between the onset of inflammation, autoantibodies and symptoms in the pre-RA phase?
3. In which persons or circumstances is the presence of RA-related autoantibodies not harmful and not associated with progression towards disease and in which persons or circumstances are RA-related autoantibodies detrimental, indicating a very early phase of RA?
4. What is the predictive value of the variables mentioned below in the pre-RA stages A, B, C and D for the development of RA?
    - Genetic and epigenetic variants
    - Environmental factors
    - RA-related autoantibodies
    - Serologic inflammation markers
    - Presence of certain symptoms
    - Patient characteristics
    - Imaging abnormalities
    - Histological abnormalities
5. Does preclinical inflammation occur in ACPA- and/or RF-negative RA?
6. Can we, with the help of prospective studies, adequately identify the individuals who will progress to RA in each of the pre-RA phases? (This may be challenging given the low prior chance on RA in the earliest pre-RA phases) Such risk stratification is basic to the development of dedicated preventive trials in the pre-RA phases.
7. Does treatment in the pre-RA phase prevent disease chronicity?

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Characterising arthralgia in the preclinical phase of rheumatoid arthritis using MRI

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ABSTRACT

Background

The phase of arthralgia is the earliest moment to clinically recognize patients who may develop Rheumatoid Arthritis (RA). Previous imaging studies in the arthralgia phase have shown that inflammation precedes RA development. It is unknown which symptoms/characteristics relate to subclinical joint inflammation as measured by MRI. Among all patients with arthralgia, those with clinically suspect arthralgia (CSA) are suspected to progress to arthritis according to the clinical judgement of their rheumatologists. We determined the symptoms/characteristics of patients with CSA who had inflammation on MRI.

Methods

102 patients with CSA and without clinical arthritis were included. They completed questionnaires, underwent joint counts and unilateral 1.5T MRI of MCP joints 2–4, wrist and MTP joints 1–5. Synovitis, bone marrow edema (BME) and tenosynovitis were scored according to the OMERACT rheumatoid arthritis MRI scoring system. Symptoms and signs were related to MRI inflammation (based on MRI scores in symptom-free controls; a sum of synovitis, BME and tenosynovitis scores ≥3 was considered positive). Whether certain clinical characteristics frequently occurred together with MRI inflammation was studied by partial least squares analysis.

Results

MRI was performed in 93 patients with CSA, 44% of whom had subclinical MRI inflammation. Synovitis was the most prevalent inflammatory feature on MRI (20%). Patients with MRI inflammation were older and were more frequently positive for anti-citrullinated peptide antibodies than patients without MRI inflammation ($p<0.001$ and 0.049). In PLS analysis, including 16 clinical and serological characteristics as independent variables and MRI inflammation as dependent variable, no clear clusters of patients with and without MRI inflammation were identified.

Conclusions

Subclinical inflammation as measured by MRI is present in 44% of patients with CSA. A combination of symptoms/characteristics incompletely differentiated patients with and without MRI inflammation.

INTRODUCTION

Rheumatoid arthritis (RA) has a period of preclinical disease. According to a recent EULAR study group, this period can be split into several phases namely, genetic and environmental risk factors for RA, systemic autoimmunity associated with RA, symptoms without clinical arthritis and unclassified arthritis [1]. Although genetic and serological risk factors for RA have been extensively studied [2,3], the phase of symptoms without clinical arthritis is relatively unexplored. The type of arthralgia and concomitant symptoms that are characteristic of a preclinical phase of RA have not yet been studied. Also, regularly used terms such as inflammatory arthralgia are not uniformly defined.

Early treatment of RA and thus early identification of disease is associated with improved disease outcome [4,5]. Hence, there is a need to characterize the patients with arthralgia who are actually at risk of RA in order to identify them from the many patients presenting with arthralgia to rheumatology outpatient clinics.

Previous studies on the preclinical phase of symptoms without clinical arthritis evaluated patients with any arthralgia who had an increased risk of RA because of the presence of anti-citrullinated peptide antibodies (ACPA) or rheumatoid factor (RF). Of these patients, 18–35% developed arthritis after a median follow-up of around 12 months [6–8]. As only 60% of patients with RA are ACPA-positive [9] and ACPA are also prevalent in the population who do not progress to RA [10,11], we decided to study the preclinical phase with a different approach. We started from a clinical point of view and evaluated patients presenting with recent-onset arthralgia that was, according to the rheumatologist, clinically suspected to progress to RA over time; this was called 'clinically suspect arthralgia' (CSA). Whether or not a patient had CSA was decided by the treating rheumatologist at the first visit before any laboratory results were known; having autoantibodies was not a requirement for having CSA.

It is known that systemic markers of inflammation are increased in the preclinical phase of RA [6,12–18]. Local inflammation in small joints has also been observed using different imaging technologies [19–21]. MRI is a sensitive tool and is more sensitive than physical examination to measure local inflammation [22]. It detects synovitis, bone marrow edema (BME), this is also called osteitis and tenosynovitis, and is most suitable for evaluating the earliest inflammatory changes in the small joints of patients considered potentially to be in the preclinical phase of RA [23,24].

Our ultimate aim is to identify patients with RA at the stage of having symptoms without clinical arthritis. In this study we considered the presence of local subclinical joint inflammation on MRI as a proxy for RA-at-risk. We aim to describe the characteristics of patients with CSA and to investigate in these patients the symptoms, signs and laboratory markers that are related to subclinical local inflammation visualised by MRI.

METHODS

Clinically suspect arthralgia (CSA) cohort

The CSA cohort is a population-based inception cohort started in April 2012 at the rheumatology outpatient clinic in Leiden, the Netherlands, with the aim of studying the preclinical phase of RA. The Leiden University Medical Centre is the only rheumatology referral centre in a healthcare region of 400,000 inhabitants. The inclusion criterion was the presence of arthralgia of the small joints for <1 year which, because of the character of the symptoms, was considered by the rheumatologist as being suspect to progress to arthritis over time. Thus, inclusion was essentially based on the 'gut feeling' of the rheumatologist. As it is not known which symptoms are predictive for arthritis development, no further criteria were included with regard to the type of symptoms. Importantly, when clinical arthritis was present at physical examination or another explanation for the arthralgia was likely such as Heberden's or Bouchard's nodes or tender points, the patients were not included.

The set-up of the rheumatology outpatient clinic of the Leiden University Medical Center is uniquely suited to identify patients in an early disease phase. For several years general practitioners have been encouraged to send any patient with a suspicion of arthritis to our outpatient clinic. The focus on early recognition was enhanced by the institution of an Early Arthritis *R*ecognition Clinic (EA*R*C) in 2010 [25]. Although the aim of this EA*R*C was to improve early detection of clinically detectable arthritis, it also provided the opportunity to identify patients with clinically suspect arthralgia.

At the first visit to the rheumatology outpatient clinic a senior rheumatologist or rheumatologist in training supervised by a senior rheumatologist decided, based on the findings of anamnesis and physical examination, whether a patient had clinically suspect arthralgia. After informed consent and inclusion, the rheumatologist completed questionnaires regarding the presenting symptoms (onset, character, localisation), current symptoms (inflammatory character, morning stiffness, fatigue) and medical and family histories. Patients filled out questionnaires regarding social status, smoking, alcohol use and work ability, the Health Assessment Questionnaire (HAQ), the Short-Form health survey-36 (SF-36) and the perceived stress scale. A 66-swollen joint count and 68-tender joint count (66-SJC and 68-TJC) were performed by trained research nurses. Blood samples were taken for routine diagnostic laboratory screening (including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), IgM-RF and ACPA (anti-CCP2, Eurodiagnostica, the Netherlands) and stored to determine other serum markers at a later time. An MRI was performed when no contraindications for MRI were present. Patients were followed longitudinally for the development of arthritis for at least 2 years with scheduled visits at 4, 12 and 24 months. If considered necessary (e.g., if patients noticed swollen joints), patients were seen in between the scheduled visits by their rheumatologist. At each follow-up visit, questionnaires were completed, joint counts were performed and blood samples taken. Follow-up ended earlier

when arthralgia had progressed to clinical arthritis. This study evaluated baseline data.

MRI scanning and scoring

MRI of the hand (wrist and metacarpophalangeal (MCP) joints) and forefoot (metatarsophalangeal (MTP) joints) was performed within 2 weeks of clinical assessment at the most painful or, in the case of equally severe symptoms on both sides, the dominant side. Patients were asked not to use any nonsteroidal anti-inflammatory drugs (NSAIDs) during the 24 hours before MRI. The joints were scanned with an MSK-extreme 1.5T MRI-scanner (GE, Wisconsin, USA). For the MCP joints and wrist the following sequences were acquired: coronal T1-weighted fast spin echo (FSE) and T2-weighted FSE with frequency selective fat saturation (fatsat) and, following intravenous administration of 0.1 mmol/kg gadolinium contrast, coronal and axial T1-weighted FSE fatsat. For the MTP joints, axial T1-weighted FSE and T2-weighted FSE fatsat sequences were obtained. Because of time limitations, post-contrast and coronal sequences were initially not obtained for the MTP joints. After 78 MRIs had been performed, post-contrast and coronal sequences were also performed in the feet (see online Supplementary File 2 for a detailed scan protocol).

Synovitis and BME were scored according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) rheumatoid arthritis MRI scoring system (RAMRIS) [23]. Although the RAMRIS was not developed to score MTP joints, others have previously adapted the RAMRIS to score MTP joints as well [26]. Tenosynovitis in the MCP joints and wrists was assessed as described by Haavardsholm et al [24]. Scoring was performed by one trained reader (HWvS) who was blinded to clinical data; the within-reader intraclass correlation coefficient for the total RAMRIS score was 0.94 and for the combined inflammation score was 0.91. The combined inflammation score (sum of the synovitis, BME and tenosynovitis scores) was the main measure of local subclinical MRI inflammation. The cut-off for the presence of MRI inflammation was defined using the MRI scores (scored by HWvS) of 19 symptom-free healthy subjects of comparable age and gender (mean age of controls 46 years, 79% women) who underwent MRI of the MCP, wrist and MTP joints on the dominant side, as described previously [20]. A combined inflammation score of ≥2, ≥3 and ≥4 was present in 21%, 10% and 0% of these healthy subjects, respectively. Based on this, a cut-off of ≥3 was primarily used.

Statistical analyses

MRI scores were studied after dichotomisation. Characteristics were compared using χ^2 tests, unpaired t tests and Mann-Whitney U tests as appropriate.

To explore whether a combination of certain clinical and serological characteristics frequently occurred together in patients with subclinical MRI inflammation, partial least squares discriminant analysis (PLS-DA) was used. PLS analysis does not test the statistically significance of differences between groups but is a variable reduction technique. It combines variables that frequently occur together in the so-called latent factors and presents for each factor the variance in the outcome that is explained by this factor. Ideally, one finds a few

latent factors that explain most of the observed variance. PLS-DA is the variant of PLS with a categorical outcome. Gender, age, presence of family history of RA, symptom characteristics (duration, onset, localisation, morning stiffness), 68-TJC, body mass index (BMI), smoking, ESR, CRP and the presence of IgM-RF and ACPA were included in the PLS as independent variables and the presence of MRI inflammation was included as untransformed dependent variable. Identified latent factors were plotted to explore whether there were distinguishable groups of patients which may represent patients with and without MRI inflammation.

The best cut-off point for clinically relevant MRI inflammation (associated with RA development) is not yet known and will be revealed by longitudinal studies. For this study we have used a cut-off score of ≥3 to dichotomise the group for the presence or absence of MRI-defined inflammation. In subanalyses a cut-off score of ≥4 for MRI inflammation was evaluated.

SPSS V.20.0 was used for analysis; p-values<0.05 were considered significant.

RESULTS

Clinical characteristics of patients with CSA

Between April 2012 and August 2013, 1,558 patients presented to the rheumatology outpatient clinic of the Leiden University Medical Centre with arthralgia. Of these, 102 (6.5%) were considered as being clinically suspect for progression to arthritis and included in the CSA cohort. The main reasons provided by rheumatologists to consider the arthralgia as clinically suspect were: joint pain that was worst in the early morning and improved with movement during the day; the presence of morning stiffness for ≥60 min; and a positive family history for RA. Table 1 presents the baseline characteristics of the included patients and Figure 1 shows the location of their tender joints.

MRI characteristics of patients with CSA

MRI was performed in 93 patients. For the final analysis a combined inflammation score of ≥3 was used, but the components of all the MRI characteristics are shown in Table 2 and in online Supplementary Table 1. Most individual lesions had a RAMRIS score of 1. Particularly for BME, bones with a score of 2 or 3 were rare. When evaluating the total scores for synovitis, BME and tenosynovitis separately, 52.7%, 51.6% and 35.5%, respectively, of the patients had a score of ≥1. Likewise, 20.4%, 9.7% and 10.8% had a score of ≥3 for the respective individual MRI features (Table 2). When summing the scores of all three MRI features, 41 patients (44.1%) had a combined inflammation score of ≥3 and were considered as 'MRI inflammation positive'.

Most inflammatory features were observed in the bones and joints of the wrist. Synovitis was most prevalent in the intercarpal, radiocarpal, MTP1 and MCP3 joints. BME occurred mainly in the capitate, lunate and MTP1. Tenosynovitis was most frequent in MCP3 (see online Supplementary Table 1 for a complete overview). Figure 2 presents examples of

patients with subclinical inflammation on MRI.

Table 1. Patient and symptom characteristics (n=102)

Characteristic	
Women, n (%)	74 (72.5)
Age in years, mean (SD)	43.3 (13.3)
Family history of RA, n (%)	50 (32.4)
Symptom duration	
In weeks, median (IQR)	15.1 (8.9–26.4)
≤12 weeks, n (%)	36 (36.7)
Symptom onset	
Gradual (>1 week, either continuous or waxing and waning), n (%)	80 (78.4)
Subacute (<1 week), n (%)	21 (20.8)
Character of initial symptoms*	
Pain, n (%)	95 (93.1)
Stiffness, n (%)	70 (68.6)
Functional disability, n (%)	28 (27.5)
Localisation of initial symptoms	
Small joints, n (%)	81 (79.4)
Small and large joints, n (%)	15 (14.7)
Large joints, n (%)	6 (5.9)
Localisation of initial symptoms	
Upper extremities, n (%)	68 (66.7)
Upper and lower extremities, n (%)	24 (23.5)
Lower extremities, n (%)	9 (8.8)
Localisation of initial symptoms	
Symmetrical, n (%)	73 (71.6)
Asymmetrical, n (%)	29 (28.4)
Presence of inflammatory character joint pain†, n (%)	78 (76.5)
Presence of morning stiffness ≥60 minutes, n (%)	44 (43.1)
Presence of fatigue, n (%)	27 (26.5)
HAQ median (IQR)	0.56 (0.25–0.88)
Daily use of NSAIDs, n (%)	20 (19.6)
BMI in kg/m^2 median (IQR)	25.1 (22.8–29.6)
68-TJC median (IQR)	6 (3–11)
Current smoker, n (%)	21 (20.6)
Current alcohol user§, n (%)	66 (66.7)
Autoantibody status	
ACPA- and/or IgM-RF-positive, n (%)	29 (28.4)
Only ACPA-positive (>7 U/mL), n (%)	4 (3.9)
Only IgM-RF-positive (>3.5 IU/mL), n (%)	14 (13.7)

ACPA and IgM-RF-positive, n (%)	11 (10.8)
Increased CRP (>10 mg/L), n (%)	14 (13.7)
Increased ESR (reference for age), n (%)	13 (12.7)

Symptoms were noted by rheumatologists as reported by the patients. Data on symptom duration and BMI were missing in four patients; symptom onset, intermittent symptoms present and initial localisation (upper, upper and lower, lower, symmetrical, asymmetrical) were missing in one patient; 68-TJC was missing in three patients; alcohol use was missing in three patients; and HAQ was missing in 24 patients. *A patient can have more than one character of the initial symptoms. †Defined as joint pain that is worst in the early morning and improves with movement during the day. §Patients not consuming daily alcohol but only one or few beverages a week were considered as alcohol users.

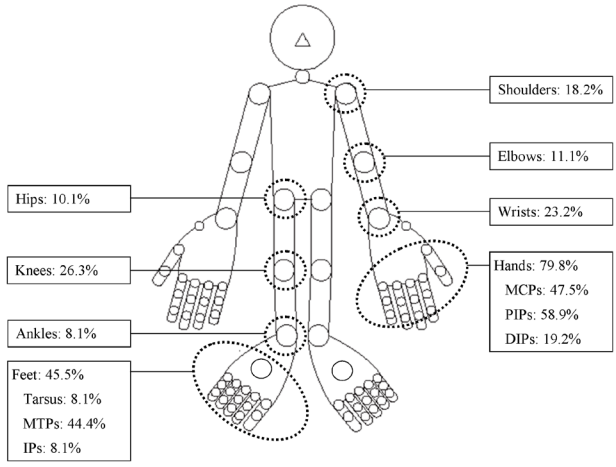

Figure 1. Localisation of tender joints in the patients with CSA without clinical arthritis. Data presented are percentages of patients; since a patient can have several tender joints, the total exceeds 100%. The median (IQR) 68-tender joint count was 6 (3–11).

Comparison of patient characteristics and subclinical MRI inflammation

Characteristics were compared between patients with (n=41) and without subclinical MRI inflammation using the predefined cut-off score of ≥3 (n=52) (Table 3). Patients with MRI inflammation were significantly older (mean 50.4 years vs 37.9 years, p<0.001) and more frequently ACPA-positive (22.0% vs 7.7%, p=0.049). Patients with MRI inflammation also had a non-significant tendency to more frequently have a subacute symptom onset, symptom onset in both small and large joints and in the lower extremities, morning stiffness ≥60 min, a higher BMI, a lower 68-TJC and an increased ESR.

Clustering of variables

Subsequently, to identify whether a combination of symptoms, signs and serological markers could distinguish the subsets of patients with and without subclinical MRI inflammation, a PLS analysis was performed with the presence of MRI inflammation as the outcome. Two latent factors were found that together explained 42.0% of the observed variance in MRI inflammation. The major important variables in these latent factors are patient groups were observed, although a slight tendency to some clustering was noted (Figure 3).

Table 2. Frequencies of MRI-features in patients with CSA, assessed using the RAMRIS dichotomised at several cut-off points

	≥1	≥2	≥3	≥4
Synovitis score				
All joints	49 (52.7%)	28 (30.1%)	19 (20.4%)	9 (9.7%)
MCP joints	20 (21.5%)	7 (7.5%)	5 (5.4%)	4 (4.3%)
Wrist	35 (37.6%)	20 (21.5%)	5 (5.4%)	2 (2.2%)
MTP joints	20 (21.5%)	4 (4.3%)	2 (2.2%)	2 (2.2%)
BME score				
All joints	48 (51.6%)	22 (23.7%)	9 (9.7%)	3 (3.2%)
MCP joints	5 (5.4%)	1 (1.1%)	-	-
Wrist	38 (40.9%)	12 (12.9%)	5 (5.4%)	2 (2.2%)
MTP joints	17 (18.3%)	4 (4.3%)	1 (1.1%)	1 (1.1%)
Tenosynovitis score*				
All joints	33 (35.5%)	16 (17.2%)	10 (10.8%)	5 (5.4%)
MCP joints	25 (26.9%)	10 (10.8%)	6 (6.5%)	4 (4.3%)
Wrist	17 (18.3%)	8 (8.6%)	4 (4.3%)	1 (1.1%)
MTP joints	NA	NA	NA	NA
Combined inflammation score†				
All joints	71 (76.3%)	55 (59.1%)	41 (44.1%)	27 (29.0%)
MCP joints	33 (35.5%)	19 (20.4%)	9 (9.7%)	9 (9.7%)
Wrist	56 (60.2%)	39 (41.9%)	26 (28.0%)	13 (14.0%)
MTP joints	30 (32.3%)	12 (12.9%)	3 (3.2%)	3 (3.2%)

* Not assessed in the feet. †Sum of synovitis, BME and tenosynovitis scores. Median total synovitis, BME, tenosynovitis and combined inflammation scores were all low, respectively 1 (IQR 0-2), 1 (IQR 0-1), 0 (IQR 0-1) and 2 (IQR 1-4). The potential range for the MRI scores according to RAMRIS are 0-36 for synovitis, 0-54 for tenosynovitis, 0-99 for BME and 0-189 for combined inflammation. NA=not assessed.

Subanalyses

MRI inflammation was defined as a combined inflammation score of ≥3. Because this cut-off is arbitrary, analyses were repeated with a cut-off score of ≥4. Patients with a combined inflammation score of ≥4 were significantly older (p<0.001), more often had morning stiffness ≥60 min (p=0.030), a lower 68-TJC (p=0.047) and more often had increased CRP (p=0.007) and ESR levels (p=0.003) than patients with a score of <4 (see online Supplementary Table 3). In PLS analysis, two latent factors were found that together explained 34.5% of the variance when MRI inflammation was defined as a combined inflammation score of ≥4. The patients' scores of these two factors are shown in online Supplementary Figure 1. No clear clusters were observed.

Follow-up of MRI-defined inflammation

To date, 29 patients with MRI inflammation have been followed for at least 4 months. Although this follow-up duration is short, 10 of these patients (34.5%) developed arthritis.

These patients had a median combined inflammation score of 4 (range 3-20). The patients were diagnosed with RA (n=8), unclassified arthritis (n=1) and psoriatic arthritis (n=1).

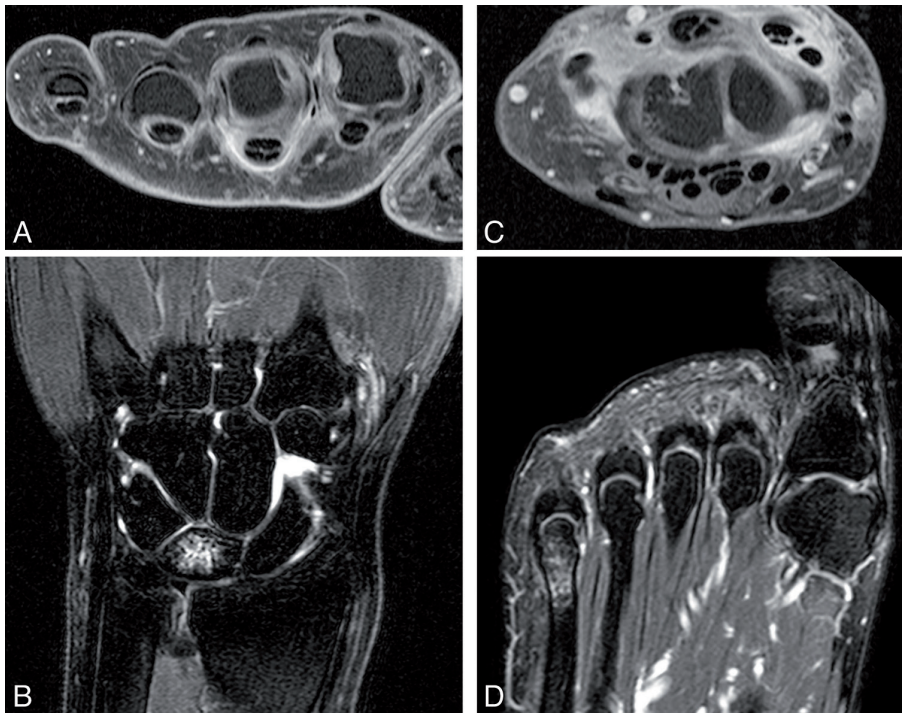

Figure 2. Subclinical inflammation shown on MRIs of MCP joints (A), wrist (B, C) and MTP) joints (D) of patients with CSA without clinically detectable arthritis. These images belong to four different patients. (A) Post-contrast axial T1-weighted FSE image with fat saturation of the MCP joints showing enhancement of the sheaths of the flexor tendons of MCP3 and MCP4, consistent with tenosynovitis. Synovitis is also present in the MCP2 and MCP3 joints. (B) Coronal T2-weighted FSE image with fat saturation of the wrist showing high signal intensity in the intercarpal joints (with enhancement on the post-contrast sequence, consistent with synovitis) and BME in the lunate. (C) Post-contrast axial T1-weighted FSE image with fat saturation of the wrist showing intercarpal synovitis and tenosynovitis of the extensor compartments 2, 5 and 6. (D) Axial T2-weighted FSE image with fat saturation of the MTP joints with BME in the head of the fifth metatarsal bone. No synovitis is detected. Patient C developed clinically detectable arthritis in this wrist during follow-up.

DISCUSSION

In the present study we aimed to describe clinical and MRI characteristics of patients with CSA. We observed that subclinical MRI inflammation was present in 44% of all patients and that 35% of these patients with CSA had already progressed to clinical arthritis within at least 4 months of follow-up. The studied symptoms and characteristics could not clearly differentiate between patients with CSA with and without MRI inflammation.

Our approach was to select patients with arthralgia who were, according to the rheumatologist, suspected of progressing to arthritis over time. Since the symptomatic phase without arthritis in the pre-RA phase is not uniformly explicated and it is not known what

Table 3. Clinical characteristics of patients with CSA without and with subclinical inflammation on MRI

	Subclinical MRI inflammation* present (n=41)	Subclinical MRI inflammation* absent (n=52)	p-value†
Women, n (%)	29 (70.7)	39 (75.0)	0.64
Age in years, mean (SD)	50.4 (13.7)	37.9 (11.1)	<0.001
Family history of RA, n (%)	12 (29.3)	18 (34.6)	0.58
Symptom duration			
In weeks, median (IQR)	14.8 (8.4-30.9)	14.6 (8.9-26.4)	0.90
≤12 weeks, n (%)	17/40 (42.5)	17/49 (34.7)	0.45
Symptom onset			0.12
Gradual, n (%)	29 (70.7)	43/51 (84.3)	
Subacute, n (%)	12 (29.3)	8/51 (15.7)	
Localisation of initial symptoms			0.12
Small joints, n (%)	31 (75.6)	45 (86.5)	
Large joints, n (%)	2 (4.9)	4 (7.7)	
Both, n (%)	8 (19.5)	3 (5.8)	
Localisation of initial symptoms			0.15
Upper extremities, n (%)	28 (68.3)	37 (71.2)	
Lower extremities, n (%)	6 (14.6)	2 (3.8%)	
Both, n (%)	7 (17.1)	13 (25.0)	
Symmetrical localization of initial symptoms, n (%)	28 (68.3)	36/51 (71.4)	0.81
Presence of inflammatory character joint pain‡, n (%)	29 (70.7)	41 (78.8)	0.37
Presence of morning stiffness			
In minutes, median (IQR)	60 (15-90)	45 (15-90)	0.34
≥60 minutes, n (%)	21 (51.2)	18 (34.6)	0.11
BMI, median (IQR) in kg/m^2	25.7 (23.1-30.3)	24.5 (21.8-27.8)	0.089
68-TJC, median (IQR)	4 (2.5-9.0)	6 (3-14.3)	0.10
Current smoker, n (%)	8 (19.5)	11 (21.2)	0.85
Current alcohol user§, n (%)	27/40 (67.5)	33/50 (66.0)	0.88
ACPA-positivity, n (%)	9 (22.0)	4 (7.7)	0.049
IgM-RF-positivity, n (%)	12 (29.3)	10 (19.2)	0.26
Increased CRP (>10 mg/L), n (%)	7 (17.1)	4 (7.7)	0.16
Increased ESR (reference for age), n (%)	7 (17.1)	3 (5.8)	0.081

*Subclinical MRI inflammation is defined as a combined inflammation score (sum of synovitis, BME and tenosynovitis scores) ≥3. †Calculated with the χ^2, unpaired t or Mann-Whitney U tests as appropriate. ‡Defined as joint pain that is worst in the early morning and improves with movement during the day. §Patients not consuming daily alcohol but only one or few beverages a week were considered as alcohol users. Data on symptom duration and BMI were missing in 4 patients; symptom onset and symmetrical localization in 1 patient; 68-TJC in 2 patients and alcohol use in 3 patients.

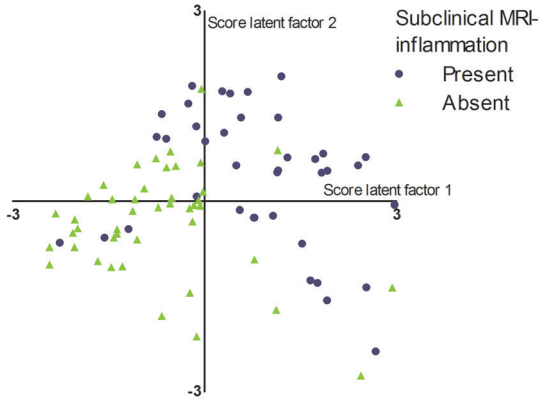

Figure 3. Clustering of variables in patients with clinically suspect arthralgia related to inflammation on MRI. In this plot each dot indicates a single person. Latent factor scores indicate how strongly each factor is represented in each patient. A dot indicates how much the variance in an individual patient is being described by latent factor 1 on the x-axis in relation to latent factor 2 on the y-axis. Patients with a combined inflammation score of ≥3 are considered as having subclinical MRI inflammation and are represented by a circle, whereas patients with a combined inflammation score of <3 who are considered as having no MRI inflammation are represented by a triangle. No clearly distinguishable groups were observed, although a tendency to some clustering was observed to discern patients with and without MRI inflammation. Patients with MRI inflammation were older and more frequently had a subacute symptom onset, initial localisation in the large joints, initial localisation in the lower extremities and morning stiffness ≥60 min, a lower 68-TJC, a higher BMI and more often had increased ESR and positivity for ACPA and IgM-RF compared with patients without MRI inflammation.

type of arthralgia is specific for the pre-RA phase, we decided to depend on the 'gut feeling' (clinical expertise) of trained rheumatologists to select patients who were suspected as being in a preclinical phase of RA. Whether or not a patient had CSA was determined at the first visit before any blood tests had been performed, and thus did not depend on the knowledge of the autoantibody status of the patient. This concept is different from that used in studies on the preclinical phase that select persons with an increased risk of RA because of the presence of autoantibodies [7] or a positive family history [27]. The advantage of the current CSA approach is that it is in line with clinical practice where patients present with certain symptoms and the decision to perform additional investigations is based on the clinical presentation. Furthermore, it may allow identification of ACPA-negative RA in the preclinical phase.

The present study is the first large study to use dedicated MRI in patients at risk of RA. Our finding that subclinical inflammation as defined by MRI is present in 44% of patients with CSA is to some extent in line with the results of previous smaller studies. A previous MRI study among 22 patients with ACPA-positive arthralgia showed higher MRI inflammation scores in these patients compared with controls [20]. Subclinical inflammation has also been visualised by positron emission tomography and ultrasonography [19,21].

The joints and bones mostly affected by MRI-defined inflammation in our CSA cohort were locations where MRI inflammation is observed in patients with early arthritis patients (MCP3 joint, capitate and lunate, radiocarpal and intercarpal joints) [22]. This strengthens the

indication that the inflammation observed in the patients with CSA in our study might be a precursor of clinical arthritis. The patients with CSA also frequently had inflammation in the MTP1 joint; this joint showed inflammation most frequently in the symptom-free controls (26%) and presumably inflammation here is not specific for RA.

Since MRI is a sensitive imaging technique, a relevant issue is which scores are normal and which reflect pathology. Several MRI studies on a small number of healthy volunteers showed MRI abnormalities to some extent [20,28,29]. Due to different scoring methods and different readers used, the data are difficult to compare. An advantage of the present study is that it included MRIs of 19 symptom-free controls of comparable age and gender to the patients with CSA. Based on our impression that a score of 1 or 2 is rather minimal and that a score of ≥3 was observed in only 10% of controls, this cut-off was used to define the presence of MRI inflammation. Because we were aware that this cut-off is rather arbitrary and none of the controls had a score of ≥4, sensitivity analyses were done with a score of ≥4 as the definition of MRI inflammation. This showed similar results, although morning stiffness, TJC and the acute phase reactants were then also significantly associated with MRI inflammation. Ultimately, longer follow-up is needed to study the conversion to clinical arthritis. This will also reveal which cut-off of MRI-defined inflammation is associated with progression to clinical arthritis and RA.

This study has limitations. Because of time limitations we initially chose not to perform coronal sequences (perpendicular to the axis of the metatarsals) and post-contrast images of the MTP joints. Synovitis of the MTP joints was therefore initially assessed without contrast enhancement on axial sequences. Although previous studies have reported that eliminating contrast affected the reliability of synovitis scoring compared with contrast-enhanced MRI, the sensitivity was reported to be high (78–90%) and the specificity moderate (31–79%) [30,31]. After 78 MRIs the scanning protocol was changed and coronal and post-contrast sequences of the foot were included, so synovitis in the MTP joints could be as reliably scored as in the wrist and MCP joints. As a consequence of the moderate specificity of non-contrast sequences, the synovitis scores of the MTP joints of the patients scanned by the first protocol might have been overestimated. On the other hand, due to lack of coronal sequences in the first protocol, the synovitis scores of the MTP joints might have been underestimated. However, synovitis of the MTP joints made a relatively small contribution to the total MRI inflammation score in the present data. A second limitation is the number of patients. Despite the infrastructural investments at our department to identify arthralgia patients early, the large majority of patients with arthralgia who presented at our outpatient clinic did not have CSA. Larger studies are needed to increase our understanding of the processes driving progression of subclinical inflammation in the pre-RA phases.

In the present study subclinical MRI inflammation was considered as proxy for RA-at-risk. Whether all patients with MRI inflammation will eventually develop arthritis is uncertain and unlikely. This will be studied during subsequent follow-up.

In conclusion, the preclinical phase of RA 'symptoms without clinical arthritis' was investigated by studying patients with CSA. Subclinical inflammation on MRI was observed in 44% of these patients. A combination of symptoms/characteristics incompletely differentiated patients with and without MRI inflammation. Follow-up will show which characteristics relate to the development of RA.

SUPPLEMENTARY DATA

Supplementary data are published on the website of the *Annals of the Rheumatic Diseases*.

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Subclinical inflammation on MRI of hand and foot of ACPA-negative arthralgia patients at risk for rheumatoid arthritis

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ABSTRACT

Introduction

It is known that anti-citrullinated peptide antibody (ACPA)-positive rheumatoid arthritis (RA) has a preclinical phase. Whether this phase is also present in ACPA-negative RA is unknown. To determine this, we studied ACPA-negative arthralgia patients who were considered prone to progress to RA for local subclinical inflammation observed on hand and foot magnetic resonance imaging (MRI) scans.

Methods

We studied a total of 64 ACPA-negative patients without clinically detectable arthritis and with arthralgia of the small joints within the previous 1 year. Because of the character of the patients' symptoms, the rheumatologists considered these patients to be prone to progress to RA. For comparisons, we evaluated 19 healthy, symptom-free controls and 20 ACPA-negative RA patients, who were identified according to the 1987 ACR criteria. All participants underwent MRI of unilateral wrist, metacarpophalangeal and metatarsophalangeal joints. Synovitis and bone marrow edema (BME) were scored according to the OMERACT rheumatoid arthritis magnetic resonance imaging scoring system, and the scores were summed to yield the 'MRI inflammation score'. Scores were compared between groups. Among the ACPA-negative arthralgia patients, MRI inflammation scores were related to C-reactive protein (CRP) levels and the tenderness of scanned joints.

Results

MRI inflammation scores increased progressively among the groups of controls and ACPA-negative arthralgia and RA patients (median scores 0, 1 and 10, respectively; $p< 0.001$). The MRI inflammation scores of ACPA-negative arthralgia patients were significantly higher than those of controls ($p=0.018$). In particular, the synovitis scores were higher in ACPA-negative arthralgia patients ($p=0.046$). Among the ACPA-negative arthralgia patients, inflammation was observed predominantly in the wrist (53%). The synovitis scores were associated with CRP levels ($p=0.007$) and joint tenderness ($p=0.026$). Despite the limited follow-up duration, five patients developed clinically detectable arthritis. These five patients had higher scores for MRI inflammation ($p=0.001$), synovitis ($p=0.002$) and BME ($p=0.003$) compared to the other patients.

Conclusion

Subclinical synovitis was observed in the small joints of ACPA-negative arthralgia patients, and especially in patients whose conditions progressed to clinically detectable arthritis. This finding suggests the presence of a preclinical phase in ACPA-negative RA. Further longitudinal studies of these lesions and patients are required to confirm this hypothesis.

INTRODUCTION

Early recognition of rheumatoid arthritis (RA) and early treatment initiation of it have been proven to be effective in reducing the disease burden over time [1,2]. For the past few years, interest in the early disease phase has also covered the preclinical phase of RA [3]. It has been shown that RA-specific autoantibodies [4,5] and serologic inflammatory markers are increased months to years before development of RA [6,7]. Also, subclinical inflammation locally in the small joints of autoantibody-positive arthralgia patients without clinical arthritis was visualised using ultrasonography, positron emission tomography (PET) and magnetic resonance imaging (MRI) [8–10]. Previous studies that investigated the preclinical phase of RA mainly or solely focused on anti-citrullinated peptide antibody (ACPA) positive RA. Consequently, it is not known whether ACPA-negative RA also has a preclinical phase. Nonetheless, up to half of all patients in early RA cohorts are ACPA-negative [1,11,12].

MRI is a suitable modality for studying early inflammatory changes in the small joints of patients in the preclinical phase of RA. It detects synovitis and is the only imaging modality that depicts bone marrow edema (BME), an MRI feature that is strongly associated with disease progression [13–15]. The availability of dedicated MRI scanners has increased the accessibility and comfort of MRI scanning. Additionally, the presence of a validated scoring methodology (the Outcome Measures in Rheumatology Clinical Trials (OMERACT) rheumatoid arthritis magnetic resonance imaging scoring system (RAMRIS)) allows comparison of the extent and severity of MRI features for research purposes [15].

In the present study, we used MRI of the hand and foot to evaluate whether ACPA-negative RA, like ACPA-positive RA, has a preclinical phase with local inflammation in small joints. Persons with any type of arthralgia are prevalent in the general population and at rheumatologic outpatient clinics. Because the majority of arthralgia patients are ACPA-negative and will never develop RA, it is challenging to identify the ACPA-negative arthralgia patients that might be in a preclinical phase of RA. We studied ACPA-negative patients without clinical arthritis and with recent-onset arthralgia of small joints who, because of the character of their symptoms, were considered prone to have disease likely to progress to RA by the treating rheumatologists. For comparisons, healthy controls and ACPA-negative RA patients were also studied.

METHODS

Participants

Three groups of participants were studied. The first group consisted of 64 ACPA-negative arthralgia patients recruited at the Leiden University Medical Centre between April 2012 and June 2013. The rheumatologists were requested to include patients who presented to the outpatient clinic without clinical arthritis upon physical examination but with arthralgia of the hand or foot joints of less than 1 year's duration of a type that was considered to have

an increased chance to progress to RA. This suspicion was based on symptoms and signs, combined with the gut feelings of the rheumatologists. Hence, based on the rheumatologists' clinical impression, these patients were considered to be in a preclinical phase of RA. The rheumatologists were encouraged to include patients whom they had otherwise also followed and not discharged because they were concerned that these patients had an increased risk for RA development. Because no type of arthralgia has yet been defined to be specific for the preclinical phase of RA, we could not assign more specific criteria with regard to the type of arthralgia patients to be included. Importantly, when another explanation for the patients' arthralgia was more likely, such as fibromyalgia, osteoarthritis or an inflammatory rheumatic disease, these patients were not included. In our present study, among all patients with arthralgia, the 64 patients who tested negative for ACPA (anti-CCCP2, Euro Diagnostica AB, Nijmegen, the Netherlands) were selected. The second group comprised 20 ACPA-negative patients who met the 1987 ACR criteria for RA [16]. These patients were included in the Leiden Early Arthritis Clinic cohort between August 2010 and July 2012. The third group consisted of 19 healthy controls without joint symptoms. Written informed consent was obtained from all participants. Approval of the study protocol was obtained from the local Medical Ethics Committee of the Leiden University Medical Centre.

Magnetic resonance imaging

All participants underwent MRI of the wrist, metacarpophalangeal (MCP) joints and metatarsophalangeal (MTP) joints with an ONI MSK Extreme 1.5T MRI scanner (GE Healthcare Life Sciences, Madison, WI, USA). In the arthralgia and RA patients, MRI of the most painful side was performed within 2 weeks after the first visit. In cases of equally severe symptoms on both sides, the dominant side was scanned. Patients were asked not to use any nonsteroidal anti-inflammatory drugs (NSAIDs) during the 24 hours before undergoing MRI. The healthy symptom-free controls underwent MRI of the dominant side. The following sequences were acquired for MCP joints and wrists: a coronal T1-weighted fast spin echo (FSE) sequence, a coronal T2-weighted FSE sequence with fat saturation and, after intravenous gadolinium contrast enhancement (0.1 mmol/kg), coronal and axial T1-weighted FSE sequences with fat saturation. Axial T1-weighted FSE sequences and T2-weighted FSE sequences with fat saturation of MTP joints were acquired. Owing to time constraints, post-contrast-enhanced images were not obtained of the MTP joints. For ethical reasons, contrast agents were not administered in controls. Synovitis and BME were scored quantitatively according to the OMERACT RAMRIS system [15]. The sum of the synovitis and BME scores yielded the 'MRI inflammation score'. Scoring was performed by one trained reader, 47% of the scans were read twice and the within-reader intraclass correlation coefficient for the MRI inflammation score was 0.91.

Analyses

Comparisons were made using a Mann-Whitney U test, Kruskal-Wallis test or χ^2 test as

appropriate. In the ACPA-negative arthralgia patients, linear regression analyses were used to study whether C-reactive protein (CRP) level was associated with MRI-determined inflammation scores. The associations between tenderness and degree of inflammation observed on MRI scans were tested by performing generalised estimating equations. This model took into account that, in every patient, ten joints were assessed. The unstructured correlation matrix was used. SPSS version 20.0 software (SPSS, Chicago, IL, USA) was used for calculations. P-values <0.05 were considered significant.

RESULTS

ACPA-negative arthralgia patients prioritised by the rheumatologists

The rheumatologists were requested to state the primary reasons why they assumed that the arthralgia patients had an increased risk for RA development. The main reasons provided were joint pain that was worst in the early morning and improved with movement during the day (thus making it an inflammatory type of arthralgia), the presence of morning stiffness of ≥60 minutes and/or a positive family history of RA. The baseline characteristics of the ACPA-negative arthralgia patients, as well as those of the ACPA-negative RA patients and symptom-free controls, are presented in Table 1. The ACPA-negative arthralgia patients who were considered at risk for progression to RA had a mean age of 42 years, and 72% were

Table 1. Patient characteristics

Characteristics	Symptom-free controls	ACPA-negative arthralgia	ACPA-negative RA
	(n = 19)	(n = 64)	(n = 20)
Mean age, yr (SD)	46.2 (11.8)	41.9 (14.3)	58.7 (14.5)
Females, n (%)	15 (78.9)	46 (71.9)	11 (55.0)
Positive family history of RA, n (%)	N/A	25 (39.1)	4 (20.0)
Median symptom duration at time of inclusion, wk (IQR)	N/A	13.4 (8.4 to 26.4)	17.6 (11.5 to 25.9)
Gradual symptom onset, n (%)	N/A	48 (75.0)	12 (60.0)
Initial symptom localisation, n (%)	N/A		
Upper extremities, n (%)		47 (73.4)	10 (50.0)
Lower extremities, n (%)		2 (3.1)	4 (20.0)
Upper and lower extremities, n (%)		15 (23.4)	6 (30.0)
Symmetrical localisation, n (%)	N/A	46 (71.9)	13 (65.0)
Median morning stiffness, min (IQR)	N/A	45 (15 to 90)	120 (30 to 120)
Median tender joint count in 68 joints (IQR)	0	5.5 (3 to 10.8)	12 (4.8 to 17.8)
Median swollen joint count 66 joints (IQR)	0	0	6 (4 to 11)
ACPA positivity (>7.0 IU/ml), n (%)	N/A	0	0
IgM RF positivity (>3.5 IU/ml), n (%)	N/A	9 (14.1)	3 (15.0)
Increased CRP level (>10 mg/L), n (%)	N/A	10 (15.6)	11 (55.0)

N/A=not applicable

female. The symptoms of most patients had started gradually (75%) and initially involved the upper extremities (73%). Tender joints were localised predominantly in the proximal interphalangeal (PIP) joints (60%) and the MCP joints (52%). Nine patients (14%) were rheumatoid factor (RF)-positive.

MRI findings in the three groups

The median (interquartile range (IQR)) MRI inflammation scores in symptom-free controls, ACPA-negative arthralgia patients and ACPA-negative RA patients were 0 (0 to 1), 1 (1 to 3) and 10 (10 to 16), respectively (p<0.001) (Figure 1).

MRI findings in ACPA-negative arthralgia patients and symptom-free controls

The ACPA-negative arthralgia patients were compared with the symptom-free controls (Figure 1). Eight (42.1%) of the nineteen symptom-free controls and forty-four (68.8%) of the sixty-four ACPA-negative arthralgia patients had any sign of inflammation based on MRI (inflammation score ≥1) (p=0.035). The median MRI inflammation scores were significantly higher in the ACPA-negative arthralgia patients than in controls (p=0.018). Subsequently, synovitis and BME scores were evaluated separately. This analysis showed that synovitis scores were significantly higher in ACPA-negative arthralgia patients than in controls (p=0.046), in contrast to BME patients (p=0.20) (Figure 1). Thus, compared to controls, patients with ACPA-negative arthralgia in particular had higher subclinical synovitis scores of small joints.

The proportion of patients with any sign of inflammation (synovitis and/or BME) on MRI in the wrist, MCP joints and MTP joints were, respectively, 53.1%, 20.3% and 31.3%. Synovitis was observed predominantly in the intercarpal (29.7%), radiocarpal (21.9%), MTP1 (17.2%) and MCP3 joints (14.1%). BME was most often present in the capitate (20.3%), lunate (15.6%) and MTP1 joints (15.6%). Figure 2 shows examples of inflammation visualised on MRI scans.

Evaluation of rheumatoid factor in ACPA-negative patients

Subsequently, the ACPA-negative arthralgia patients were assigned to RF-positive (n=9) and RF-negative (n=55) groups. No differences in MRI inflammation, synovitis and BME scores were observed (p=0.63, 0.62 and 0.90, respectively) (Additional file 1: Figure S1). We observed similar differences when the ACPA-negative RA patients were stratified.

Evaluation of traditional measures of inflammation in ACPA-negative arthralgia patients

Furthermore, we evaluated whether the degree of inflammation visualised on MRI scans of ACPA-negative arthralgia patients was associated with the level of serological inflammation as measured by CRP levels. The synovitis score was significantly associated with CRP level (β=0.10, p=0.007), indicating that each 1 mg/L increase in CRP level resulted in a 0.10 increase in synovitis score. The BME score was not associated with CRP level (p=0.88). Also, the MRI-based inflammation score was not significantly associated with CRP level (β=0.10,

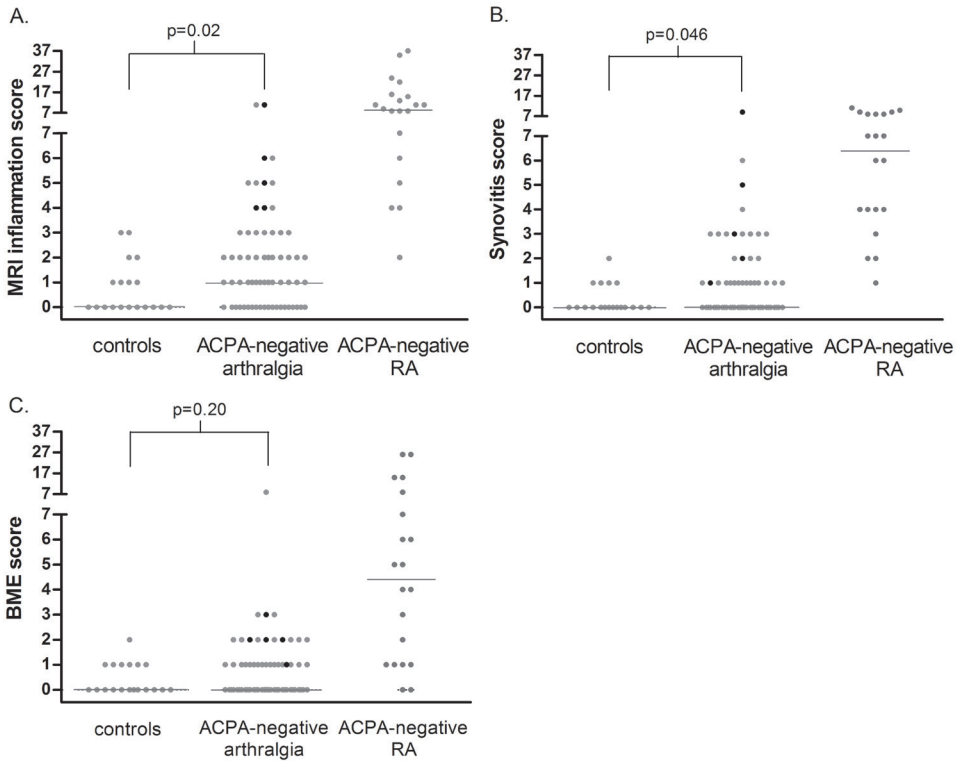

Figure 1. MRI-based inflammation scores shown separately for the three study groups. (A) MRI inflammation scores (synovitis plus bone marrow edema (BME)). (B) Synovitis scores. (C) Bone marrow edema scores. The three study groups are the symptom-free controls, the ACPA-negative arthralgia patients and the ACPA-negative RA patients, based on the 1987 criteria for RA [16]. The scores presented are for all participants individually (dots) and the median scores per group (horizontal lines). The black dots indicate the ACPA-negative patients who developed clinically detectable arthritis during the median follow-up of 9 months. The y-axes are split because RA patients had higher scores than the symptom-free controls and ACPA-negative arthralgia patients. The presented p-values were obtained by comparing the scores of ACPA-negative arthralgia patients and symptom-free controls. All p<0.001 for differences in MRI-based inflammation, synovitis and BME scores between the three groups. BME=bone marrow edema.

p=0.066).

Subsequently, we studied whether tender joints had higher MRI-based scores than non-tender joints. The presence of joint tenderness was significantly associated with synovitis score (p=0.026, OR=1.15), indicating that tender joints had 15% higher odds on one point increase in synovitis score compared to non-tender joints. No significant associations were observed between joint tenderness and BME scores (p=0.18) or MRI-based inflammation scores (p=0.53).

Follow-up of ACPA-negative arthralgia patients

The follow-up duration of the ACPA-negative arthralgia patients was still limited at a median

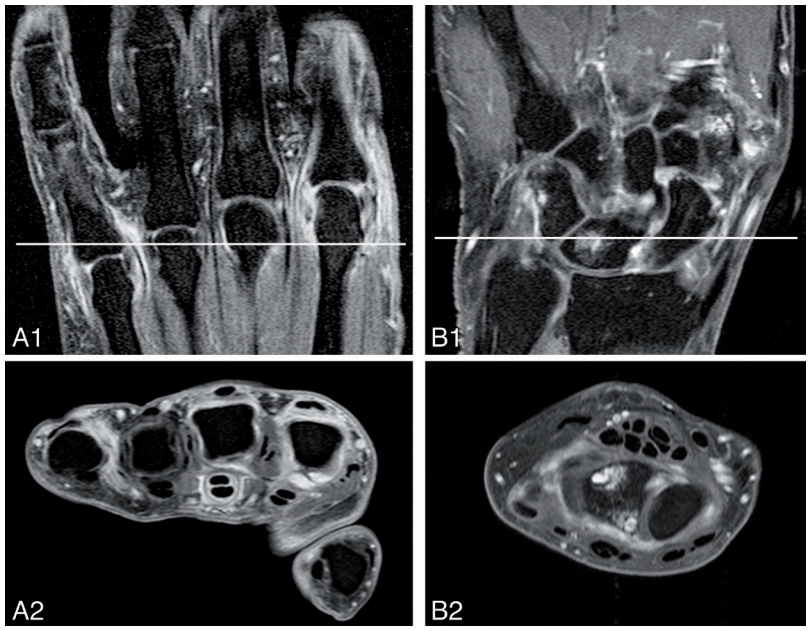

Figure 2. Subclinical inflammation visualised by MRI of MCP joints and wrists of two different ACPA-negative arthralgia patients without clinically detectable arthritis. The white lines in the top coronal images reflect the localisation of the bottom axial images. (A) Post-contrast enhancement coronal (A1) and axial (A2) T1-weighted FSE images with fat saturation showing enhancement of the MCP2, MCP3 and MCP5 joints, which is consistent with active synovitis. Also, pronounced tenosynovitis in the third flexor tendon is present, although tenosynovitis is not included in the OMERACT RAMRIS score and was not evaluated in the present study. This patient developed clinically detectable arthritis during follow-up. (B) Post-contrast enhancement coronal (B1) and axial (B2) T1-weighted FSE images with fat saturation showing bone marrow edema and erosions (confirmed on the pre-contrast enhancement T1-weighted FSE sequence) in the lunate. Also, there is active synovitis in the intercarpal joint.

of 9 months (IQR 5 to 11). During this period, five of the ACPA-negative arthralgia patients developed clinical arthritis as detected by their rheumatologists during physical examinations (7.8%). Median (IQR) scores for MRI-based inflammation, synovitis and BME for these patients were, respectively, 5 (4 to 8.5), 3 (1.5 to 7) and 2 (1.5 to 2.5) (Figure 1). These scores were significantly higher than those of the ACPA-negative arthralgia patients who did not or had not yet developed clinical arthritis (inflammation: p=0.001; synovitis: p=0.002; and BME: p=0.003). Of the five patients who developed clinical arthritis, three were diagnosed with RA, one with unclassified arthritis and one with psoriatic arthritis. At the time of clinical arthritis development, all patients were still ACPA-negative.

DISCUSSION

Early intervention in RA is associated with a more favorable disease course [1,2]. The recognition that systemic inflammatory markers are increased in the preclinical phase [6,7] and that inflammation is also locally present in small joints has increased interest in investigation of the preclinical phase of RA [8–10]. The ultimate hope is that intervention in the preclinical phase

will prevent the development of the classical picture of RA. The large majority of studies on the preclinical phase have focused on patients with ACPA [3]. To the best of our knowledge, this study is the first to assess whether local subclinical inflammation is also present in ACPA-negative pre-RA patients. We observed that ACPA-negative arthralgia patients had higher MRI-based inflammation scores than healthy participants and that higher MRI-based synovitis scores were associated with higher CRP levels.

Identifying ACPA-negative arthralgia patients with an increased chance of developing RA is more challenging compared to other pre-RA studies were the presence of RA-related autoantibodies was measured and considered as a marker of increased risk. In the present study, rheumatologists were asked to select patients who, in their view, had an increased chance of developing RA. Because no type of arthralgia has yet been defined to be specific for pre-RA, we could not assign more specific criteria with regard to the type of arthralgia to be included. Retrospectively, the reasons for rheumatologists to consider patients as having an increased chance for developing RA were mainly joint pain that was worst in the early morning and improved with movement during the day (an inflammatory type of pain), the presence of morning stiffness of ≥60 minutes and a positive family history for RA. An advantage of the approach used in present study is that it resembles current clinical practice. It is of note that the studied arthralgia patients were selected from a total number of 1,335 arthralgia patients who visited our outpatient clinic between April 2012 and June 2013. The observation that 69% of the patients who were considered to have an increased chance of developing RA had any signs of subclinical inflammation on MRI scans might indicate that the rheumatologists did reasonably well in selecting ACPA-negative arthralgia patients.

The MRI inflammation scores were higher in ACPA-negative arthralgia patients than in symptom-free controls. Patients with ACPA-negative RA had much higher MRI-based inflammation scores than those in the other two groups, which was expected because these patients had clinically detectable joint inflammation. The inflammatory lesions observed in ACPA-negative arthralgia patients were small, but were located at locations that are known to be affected in RA, such as the intercarpal bones and the MCP3 and MTP1 joints [14].

Interestingly, MRI-based synovitis scores, but not BME scores, were increased in ACPA-negative arthralgia patients compared to symptom-free controls. BME is more prevalent in ACPA-positive RA patients than in ACPA-negative RA patients, and it is a strong predictor of progression of joint destruction [13,14,17]. The finding of no increase in BME score in the preclinical phase of ACPA-negative patients might suggest that BME is not an early phenomenon in ACPA-negative RA or reflects a lower prevalence of BME in ACPA-negative RA patients, a subset of RA that is also characterised by less severe radiological progression [18]. Larger and longitudinal studies are required to determine the value of BME in this disease subset.

This study has several limitations. The number of symptom-free controls studied is

relatively low. Second, for ethical reasons, the controls did not receive intravenous contrast fluid. Researchers in previous studies have suggested that eliminating contrast enhancement does not affect BME scores, although it may affect the reliability of synovitis scoring [19,20]. In studies in which MRI scans with contrast enhancement were used as the gold standard, the sensitivity for synovitis scoring on the basis of high-field MRI without contrast enhancement has been reported to be high (78% to 90%), but the specificity has been reported to be moderate (31% to 79%) [19,20]. As a consequence of the moderate specificity in this study, the scores of the symptom-free controls might have been overestimated. Consequently, the differences in synovitis scores between the arthralgia patients and the healthy controls might have been underestimated. So, although the absence of contrast enhancement in the controls is a clear limitation, the results of previous studies [19,20] indicate that the differences might have been larger in cases of contrast administration to controls. Another limitation is the short duration of follow-up, which ranged from 1 to 16 months. The present study therefore provides mainly cross-sectional data. Longer follow-up is required to determine which ACPA-negative arthralgia patients and which inflammatory lesions detected by MRI are most predictive of progression to clinically detectable arthritis. Nonetheless, it is notable that arthralgia patients who developed clinical arthritis had higher MRI-based inflammation scores. A research question that remains unanswered is the long-term course of inflammation detected on MRI scans. Serial MRI scans are needed to determine whether MRI-based inflammation is relapsing, remitting or stable over time.

CONCLUSION

ACPA-negative arthralgia patients, especially patients whose conditions progress to clinical arthritis, have subclinical inflammation visualised on MRI scans of the hand and foot, suggesting that also ACPA-negative RA has a preclinical symptomatic phase.

SUPPLEMENTARY DATA

Supplementary data are published on the website of *Arthritis, Research & Therapy*.

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Clinical factors, ACPA and MRI-detected subclinical inflammation in relation to progression from Clinically Suspect Arthralgia to arthritis

5

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ABSTRACT

Introduction

Patients with clinically suspect arthralgia (CSA) have, according to their rheumatologists, an increased risk of rheumatoid arthritis (RA), but their actual outcome is unexplored. This longitudinal study investigated (1) progression from CSA to clinically detectable arthritis and (2) associations of clinical factors, serological factors (among which are anti-citrullinated peptide antibodies (ACPA)) and MRI-detected subclinical inflammation with arthritis development.

Methods

150 patients with CSA were followed for ≥6 months. At baseline, clinical and serological data were collected and unilateral 1.5 T-MRI of metacarpophalangeal (MCP), wrist and metatarsophalangeal (MTP) joints was made. MRI scoring was done according to the RA MRI scoring system. Subclinical MRI inflammation was defined based on MRI results of 193 symptom-free persons.

Results

During follow-up (median=75 weeks, IQR=41-106 weeks), 30 patients developed clinical arthritis; 87% did so <20 weeks after inclusion. In multivariable analyses, age, localisation of initial symptoms in small and large joints (compared with small joints only), C-reactive protein level, ACPA-positivity and subclinical MRI inflammation significantly associated with arthritis development; ACPA and MRI inflammation were most strongly associated (HR (95% CI) respectively, 6.43 (2.57 to 16.05) and 5.07 (1.77 to 14.50)). After 1 year follow-up, 31% of the patients with MRI inflammation and 71% of the ACPA-positive patients with MRI inflammation had progressed to arthritis. Forty-three per cent of the patients that developed arthritis within 1 year were ACPA-negative; 78% of them had subclinical MRI inflammation at baseline. When MRI inflammation was absent arthritis development was infrequent (6% in all patients with CSA and 3% in ACPA-negative patients with CSA).

Conclusions

Subclinical MRI inflammation precedes clinical arthritis with a few months. Subclinical MRI inflammation is, independent of other factors such as ACPA, associated with arthritis development.

INTRODUCTION

There is an upcoming need to identify individuals in the very early phase of rheumatoid arthritis (RA) in which clinically apparent arthritis is not yet present. Although not proven, it is assumed that treatment initiation in this phase enables better disease modification and might contribute towards preventing arthritis becoming chronic. The first possible moment to clinically recognise patients at risk of RA is the phase of symptoms without clinically detectable arthritis [1]. The symptoms that are specific for this phase are not yet identified, but clinical expertise might be an accurate tool to select patients with arthralgia with an increased risk of RA [2]. Patients with arthralgia that, based on their symptoms and signs, have an increased risk of RA according to their rheumatologists, are indicated to have clinically suspect arthralgia (CSA) [3]. The approach to select patients on clinical grounds before ordering additional tests is in line with clinical care and allows identifying autoantibody-positive and autoantibody-negative RA in the phase before clinically detectable arthritis.

Thus far, the long-term outcome of patients with arthralgia that were clinically suspect for progression to RA has not been investigated extensively. Moreover, the value of risk factors or tests in patients with CSA is unexplored. Two previous studies on patients with unspecified arthralgia or aspecific musculoskeletal symptoms who had RA-related autoantibodies revealed that morning stiffness, joint tenderness and (high levels of) anti-citrullinated peptide antibodies (ACPA) were associated with arthritis development [4,5]. However, the prognostic value for arthritis development of clinical and serological factors in patients with CSA is still unknown.

Also the value of advanced imaging in patients with CSA is unexplored. In a previous cross-sectional study, we observed that 44% of the patients with CSA had MRI-detected subclinical inflammation of hand and foot joints and that these patients with subclinical inflammation could not be adequately identified by presence of clinical or serological characteristics, suggesting that MRI-detected inflammation may have some diagnostic value. Though the predictive value of MRI-detected inflammation has still to be determined [3], an advantage of MRI is its sensitivity to detect inflammation [6,7]. MRI depicts synovitis, tenosynovitis and bone marrow oedema (BME), that is also called 'osteitis' in RA [8,9]. Because the specificity of MRI-detected inflammation has been studied scarcely, we recently performed MRI of hands and feet in 193 symptom-free persons [10]. These data served as reference and allowed to define MRI-detected subclinical inflammation for the present study.

In this first longitudinal study on patients with CSA, we aimed to determine (1) progression to clinically detectable arthritis, (2) the association of clinical and serological factors (among which are ACPA) with progression to clinical arthritis, (3) the association of subclinical MRI inflammation with progression to clinical arthritis and (4) whether subclinical MRI inflammation has an additive value compared with the other mentioned risk factors.

METHODS

Patients

All patients were included in the CSA cohort which is described previously in detail elsewhere [3]. This inception cohort was set up in 2012 in the Leiden University Medical Centre (Netherlands), which is the only referral centre in a healthcare population of >400000 inhabitants to study the symptomatic phase of RA without clinically detectable arthritis. Inclusion criteria were having arthralgia of the small joints for <1 year that was, according to the clinical expertise of the rheumatologist, suspected to progress to RA over time. No further criteria were made with regards to the type of symptoms and thus inclusion was essentially based on the expert opinion of the rheumatologist. Importantly, CSA was not present if clinical arthritis was observed at physical examination or another explanation for the arthralgia was more likely (eg, osteoarthritis and fibromyalgia).

At baseline, questionnaires (among others on work ability, the Health Assessment Questionnaire and Short-Form health survey-36) were completed, physical examination performed, blood obtained (among others for determination of ACPA (anti-cyclic citrullinated peptide 2 (anti-CCP2), positive if >7 U/mL, Eurodiagnostica, Netherlands) and IgM rheumatoid factor (RF) (positive if >3.5 IU/mL) and an MRI performed [3].

Patients were prospectively followed with scheduled visits at 4 months, 12 months and 24 months. If necessary (for instance when the patient experienced more symptoms or noticed a swollen joint) patients were seen in between the scheduled visits by their rheumatologist. Follow-up ended earlier when clinical arthritis had developed.

For the present study, the patients with a follow-up duration of ≥6 months were selected; these patients (n=150) were included between April 2012 and July 2014. None of the patients with CSA were treated with disease-modifying antirheumatic drugs (DMARDs) or (systemic and local) glucocorticoids prior to inclusion and during follow-up.

MRI scanning and scoring

MRI of the metacarpophalangeal (MCP)2–5, wrist and metatarsophalangeal (MTP)1–5 joints of the most painful side, or the dominant side in case of equally severe symptoms at both sides, was performed ≤2 weeks after clinical assessment. Patients were asked not to use nonsteroidal anti-inflammatory drugs (NSAIDs) during 24 h before MRI. The joints were scanned with a musculoskeletal (MSK)-extremity 1.5T-MRI scanner (GE, Wisconsin, USA) using contrast-enhancement and according to the RA MRI scoring system (RAMRIS) protocol. See online Supplementary File 1 for a detailed scan protocol.

Synovitis and BME in the MCP, wrist and MTP joints were scored according to the Outcome Measures in Rheumatology Clinical Trials (OMERACT) RAMRIS [8], the carpometacarpal (CMC)-1 joint (trapezium and base metacarpal-1) was excluded. Tenosynovitis in the wrist and MCP joints was assessed as described by Haavardsholm et al

[9]. The sum of the synovitis, BME and tenosynovitis scores was the total MRI inflammation score. Scoring was performed by two independent trained readers (HWvS, LM) blinded to clinical data. The within-reader intraclass correlation coefficients for the total MRI inflammation score were 0.99 and 0.98; the between-reader interclass correlation coefficient was 0.96. Mean scores of the two readers were studied.

At the time of analyses, MRIs were also categorised into positive or negative for subclinical MRI-detected inflammation. Frequencies of MRI-detected synovitis, tenosynovitis and BME that were observed at the same anatomical location in symptom-free persons recruited from the general population of the same age category were used as reference (see additional file in this chapter) [10]. In these symptom-free persons it was observed that MRI-detected inflammation was prevalent, especially at higher age and at preferential locations. Subclinical MRI inflammation was considered present if (1) both readers scored that joint (or bone in case of BME) positive for MRI inflammation and (2) the score obtained at a joint/bone was present in <5% of age-matched symptom-free persons. For example, subclinical MRI inflammation was present when both readers scored grade ≥1 for synovitis at MCP3 in a 30-year old patient. The MRI was considered negative if only one reader scored grade 1 and the other reader grade 0. If the patient was aged 50 years and both readers scored grade 1, the MRI was also negative as ≥5% of symptom-free persons of the same age category had also synovitis grade 1 at MCP3 [10]. The MRI results were not reported to the treating rheumatologist.

Outcome

The main outcome was development of arthritis detected at physical examination (66 joints were assessed) by the rheumatologist. If arthritis was achieved, follow-up in the CSA cohort ended. In sensitivity analyses another outcome, initiation of DMARD therapy (including steroids), was studied. Medical files of all patients were studied on these outcomes until 24 December 2014.

Statistical analyses

Cox proportional hazards regression analyses were used. Time to clinical arthritis was the time from inclusion to the date of first detection of clinical arthritis. Patients who did not develop arthritis were censored at the date of the 2 years' follow-up visit or at the date that all medical files were studied on arthritis development. Additionally, the diagnostic performance of ACPA-positivity and presence of subclinical MRI inflammation were evaluated for arthritis development at 1 year follow-up. Details on the statistical methods are presented in online Supplementary File 2).

RESULTS

Baseline clinical characteristics

One hundred and fifty patients with CSA were studied. Table 1 presents the baseline characteristics. Mean age of the studied patients was 43.2 years (SD 12.9) and 72.7% were female. The median symptom duration was 18 weeks (IQR 9-30) and 16.0% were ACPA-positive (Table 1).

Development of clinical arthritis

During follow-up one patient developed gout. This patient was excluded from further analyses as the patient did not belong to the non-arthritis group and the diagnosis was outside the spectrum of chronic arthritis/RA. The remaining 149 patients with CSA had a median follow-up duration of 75 weeks (IQR 41-106). Within this follow-up period 30 patients developed clinically detectable arthritis. At arthritis development, 23 patients were diagnosed with RA (according to the 2010 ACR/EULAR criteria), 6 with undifferentiated arthritis and 1 with psoriatic arthritis.

The median time period between inclusion and arthritis development was 7 weeks.

Table 1. Baseline clinical and MRI characteristics of all patients and separately for the patients that have and have not developed clinical arthritis during follow-up

	All patients n=150*	No arthritis during follow-up (n=119)	Arthritis during follow-up (n=30)
Clinical characteristics			
Age in years, mean (SD)	43.2 (12.9)	43.1 (12.8)	43.9 (13.7)
Female, n (%)	109 (72.7)	87 (73.1)	22 (73.3)
Family history positive for RA, n (%)	51 (34.0)	38 (31.9)	12 (40.0)
Symptom duration# in weeks, med (IQR) (n=141)	18 (9-30)	18 (10-31)	17 (8-30)
Gradual symptom onset (>1 week) (n=149)	31 (20.8)	95 (80.5)	22 (73.3)
Localisation of initial symptoms (n=149)			
Small joints, n (%)	127 (85.2)	107 (90.7)	19 (63.3)
Small and large joints, n (%)	15 (10.1)	6 (5.1)	9 (30.0)
Large joints, n (%)	7 (4.7)	5 (4.2)	2 (6.7)
Localisation of initial symptoms			
Upper extremities, n (%)	108 (72.0)	88 (73.9)	20 (66.7)
Upper and lower extremities, n (%)	28 (18.7)	21 (17.6)	7 (23.3)
Lower extremities, n (%)	14 (9.3)	10 (8.4)	3 (10.0)
Symmetrical localisation of initial symptoms, n (%) (n=149)	110 (73.8)	91 (77.1)	19 (63.3)
Morning stiffness ≥60 min at inclusion, n (%) (n=144)	53 (36.8)	38 (33.6)	15 (50.0)
68-TJC, med (IQR) (n=146)	5 (3-10)	6 (3-10)	5 (3-7.5)
BMI in kg/m2, mean (SD) (n=149)	26.6 (5.2)	26.5 (5.0)	26.7 (6.1)
Present smoker, n (%)	38 (25.3)	29 (24.4)	9 (30.0)

CRP-level in mg/L, med (IQR)	0 (0-4.6)	0 (0-4)	1.5 (0-14.5)
CRP-level >5 mg/L, n (%)	31 (20.7)	21 (17.6)	10 (33.3)
RF-positive (>3.5 IU/mL), n (%)	33 (22.0)	15 (12.6)	18 (60.0)
ACPA-positive (>7 U/mL), n (%)	24 (16.0)	8 (6.7)	16 (53.3)
MRI characteristics			
MRI categorised into positive or negative for any subclinical inflammation and for specific inflammatory features			
	All patients n=144*	No arthritis during follow-up (n=116)	Arthritis during follow-up (n=27)
Presence of any MRI-detected inflammation, n (%)	66 (45.8)	44 (37.9)	22 (81.5)
Only synovitis, n (%)	9 (6.3)	9 (7.8)	0 (0)
Only BME, n (%)	12 (8.3)	10 (8.6)	2 (7.4)
Only tenosynovitis, n (%)	15 (10.4)	7 (6.0)	8 (29.6)
Synovitis and BME, n (%)	3 (2.1)	2 (1.7)	1 (3.7)
Synovitis and tenosynovitis, n (%)	18 (12.5)	12 (10.3)	6 (22.2)
BME and tenosynovitis, n (%)	2 (1.4)	1 (0.9)	1 (3.7)
Synovitis, BME and tenosynovitis, n (%)	7 (4.9)	3 (2.6)	4 (14.8)
Presence of MRI-detected synovitis, n (%)	37 (25.7)	26 (22.4)	11 (40.7)
Presence of MRI-detected BME, n (%)	24 (16.7)	16 (13.8)	8 (29.6)
Presence of MRI-detected tenosynovitis, n (%)	42 (29.2)	23 (19.8)	19 (70.4)

38 patients (25.3%) were positive for ACPA and/or RF.
The median total RAMRIS inflammation score was 2 (IQR 1-5); the total RAMRIS scores for synovitis, BME and tenosynovitis were 1 (IQR 0-2.5), 0.5 (IQR 0-1.5) and 0 (IQR 0-1.5), respectively. Characteristics were not compared between the groups of patients that have and have not developed clinical arthritis during follow-up because the patients have different follow-up durations.
* One patient that developed gout during follow-up was excluded from further analyses as the patient did not belong to the non-arthritis group and the diagnosis was outside the spectrum of chronic arthritis/RA.
# Duration since the start of symptoms

Of all patients that progressed to arthritis, 87% had done so within 20 weeks after inclusion (Figure 1).

Clinical factors and ACPA in relation to arthritis development

In order to investigate whether baseline clinical factors and ACPA were associated with progression from CSA to clinical arthritis, univariable Cox regression analyses were performed (Table 2). An increased hazard on developing arthritis was observed for patients that presented with initial symptoms located in the small and large joints (HR=5.28 compared with small joints only (95% CI 2.38 to 11.73, p<0.001), patients with higher C reactive protein (CRP) levels (HR=1.06/mg/L, 95% CI 1.03 to 1.09, p<0.001), RF-positive patients (HR=6.94, 95% CI 3.34 to 14.43, p<0.001) and ACPA-positive patients (HR=10.07, 95% CI 4.87 to 20.82, p<0.001)). Age, presence of morning stiffness and number of tender joints were not significantly associated.

Subclinical MRI inflammation in relation to arthritis development

Table 2. Results of univariable Cox regression analyses of baseline clinical and serological factors in relation to arthritis development

	HR (95% CI)	p-value
Age, per year	1.004 (0.98 to 1.03)	0.78
Female	1.02 (0.45 to 2.29)	0.96
Family history positive for RA	1.37 (0.66 to 2.85)	0.39
Symptom duration per week (n=141)	0.99 (0.98 to 1.01)	0.32
Gradual symptom onset (n=148)	0.68 (0.30 to 1.53)	0.35
Localisation of initial symptoms (n=148)		
Small joints only	Ref	Ref
Large joints only	1.89 (0.44 to 8.14)	0.39
Small and large joints	5.28 (2.38 to 11.73)	<0.001
Localisation of initial symptoms		
Upper extremities	Ref	Ref
Lower extremities	1.36 (0.40 to 4.58)	0.62
Upper and lower extremities	1.47 (0.62 to 3.47)	0.38
Symmetrical localisation of initial symptoms (n=148)	0.59 (0.28 to 1.23)	0.16
Morning stiffness ≥60 min (n=143)	1.89 (0.92 to 3.87)	0.081
68-TJC (n=145)	0.98 (0.93 to 1.04)	0.47
BMI, per kg/m2 (n=147)	1.01 (0.94 to 1.08)	0.80
Present smoker	1.28 (0.59 to 2.79)	0.54
CRP-level, per mg/L	1.06 (1.03 to 1.09)	<0.001
RF-positive	6.94 (3.34 to 14.43)	<0.001
ACPA-positive	10.07 (4.87 to 20.82)	<0.001

Presented are the HRs of univariable analyses including 149 patients with CSA of which 30 developed clinical arthritis. When data on clinical characteristics were missing, the number of patients with available data is presented in the first column.

In six patients MRI was not performed, because of (suspected) pregnancy (n=2), metallic foreign body in biceps tendon (n=1), logistical reasons (n=2) or development of clinical arthritis ≤2 weeks after inclusion (n=1).

Table 1 presents baseline MRI characteristics. Median continuous RAMRIS scores were low (online Supplementary Table S1 presents continuous RAMRIS scores for individual joints/bones). Univariable analyses showed that higher MRI inflammation scores were associated with arthritis development (Table 3).

Then the continuous MRI inflammation scores were dichotomised. Since it was recently observed that MRI-detected inflammation is also present in the general population and depends on age, anatomical location and type of inflammation [10], these were considered when defining an 'abnormal MRI'. A joint (or bone in case of BME) was categorised as

positive for inflammation when <5% of the general population of the same age category had inflammation at this location (online Supplementary Table S2 presents frequencies of positive joints/bones for MRI-detected inflammation). Sixty-six patients with CSA (45.8%) had a positive MRI for any subclinical inflammation, indicating that at least 1 joint/bone had synovitis, BME or tenosynovitis (Table 1): 20.1% of the patients had 1 positive joint/bone, 18.8% 2-5 positive joints/bones and 6.9% ≥6 positive joints/bones (the maximum number of positive joints/bones was 24). When evaluating the individual MRI features, 25.7% of the patients had MRI-detected synovitis, 16.7% BME and 29.2% tenosynovitis (Table 1).

Univariable Cox regression analyses with arthritis development as outcome revealed that presence of any MRI-detected subclinical inflammation at baseline was associated with a six times increased hazard on arthritis (HR=6.12, 95% CI 2.32 to 16.19, p<0.001, Figure 1). In addition, the hazard on clinical arthritis increased when more joints/bones were scored positive for MRI inflammation (HR=1.23 per additional positive joint/bone, 95% CI 1.13 to 1.33, p<0.001). Evaluating the three MRI features separately showed the strongest association for MRI-detected tenosynovitis (HR=7.56), though MRI-detected synovitis and BME were also significantly associated with arthritis development (HR=2.22 and 2.39 respectively, all p<0.05, Table 3). Because synovitis, BME and tenosynovitis were frequently present in the same patient (Table 1), multivariable Cox regression analyses were done to determine which type(s) of MRI-detected inflammation were independently associated with arthritis development (Table 3). We observed that MRI-detected tenosynovitis was independently associated (HR=8.39, 95% CI 3.38 to 20.81, p<0.001) with arthritis development.

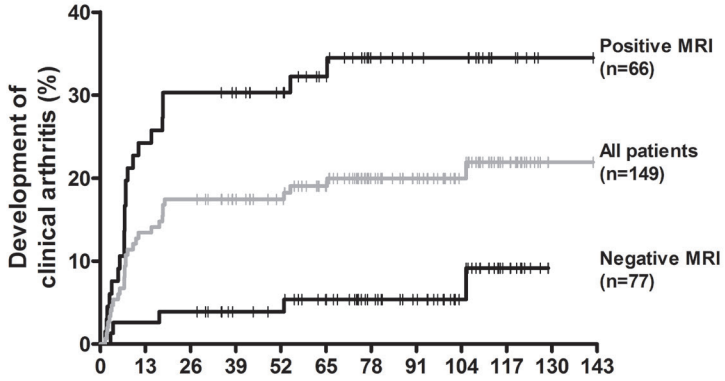

Figure 1. Development of clinical arthritis over time for all patients and for the patients with and without MRI-detected inflammation separately. Presented are the curves for development of clinical arthritis over time in all 149 patients with CSA and for the patients with a positive and a negative MRI separately (six patients did not undergo an MRI). The HR of developing arthritis with a positive MRI was 6.12 (95% CI 2.32 to 16.19, p<0.001). All patients were followed for ≥6 months (median follow-up duration 75 weeks). The vertical lines indicate that a patient is censored.

Table 3. Results of Cox regression analyses of MRI-detected subclinical inflammation at baseline in relation to arthritis development

	HR (95% CI)	p-value
Continuous RAMRIS scores		
Univariable		
Total inflammation score, per unit	1.14 (1.08 to 1.20)	<0.001
Total synovitis score, per unit	1.29 (1.14 to 1.47)	<0.001
Total BME score, per unit	1.28 (1.13 to 1.46)	<0.001
Total tenosynovitis score, per unit	1.25 (1.11 to 1.39)	<0.001
Multivariable		
Total synovitis score, per unit	1.09 (0.86 to 1.38)	0.47
Total BME score, per unit	1.20 (1.03 to 1.38)	0.016
Total tenosynovitis score, per unit	1.15 (0.94 to 1.41)	0.17
MRI dichotomised for the presence of any subclinical inflammation and for specific inflammatory features		
Univariable		
Presence of any MRI-detected inflammation	6.12 (2.32 to 16.19)	<0.001
Presence of MRI-detected synovitis	2.22 (1.03 to 4.78)	0.042
Presence of MRI-detected BME	2.39 (1.04 to 5.46)	0.039
Presence of MRI-detected tenosynovitis	7.56 (3.30 to 17.32)	<0.001
Multivariable		
Presence of MRI-detected synovitis	0.72 (0.31 to 1.69)	0.45
Presence of MRI-detected BME	2.09 (0.91 to 4.81)	0.084
Presence of MRI-detected tenosynovitis	8.39 (3.38 to 20.81)	<0.001

Presented are the HRs of analyses including 143 patients with CSA that underwent MRI of which 27 developed clinical arthritis. The HR of 1.14 for the total inflammation score indicates that when the total MRI inflammation score increased with 1 unit the hazard on arthritis development increased with a factor 1.14.

Combination of clinical factors, ACPA and subclinical MRI inflammation in relation to arthritis development

Then, we questioned if the association of subclinical MRI inflammation with arthritis development was independent of the associations of other factors (age, initial localisation of the symptoms, CRP-level, ACPA-positivity). Multivariable Cox regression analyses (Table 4) revealed an increased hazard for younger patients (HR=0.96 per year older, 95% CI 0.93 to 0.996, p=0.028), patients with initial localisation of symptoms in small and large joints (HR=4.30 compared with small joints only, 95% CI 1.70 to 10.86, p=0.002), patients with higher CRP-levels (HR=1.05/mg/L, 95% CI 1.01 to 1.09, p=0.021), ACPA-positive patients (HR=6.43, 95% CI 2.57 to 16.05, p<0.001) and patients with presence of any MRI-detected subclinical inflammation (HR=5.07, 95% CI 1.77 to 14.50, p=0.002). Similar results were obtained when including continuous total MRI inflammation scores instead of MRI positivity

Table 4. Results of multivariable Cox regression analysis of clinical and serological factors and MRI-detected subclinical inflammation at baseline in relation to arthritis development

	HR (95% CI)	p-value
Age, per year	0.96 (0.93 to 0.996)	0.028
Localisation of initial symptoms		
Small joints only	Ref	Ref
Large joints only	2.35 (0.41 to 13.61)	0.34
Small and large joints	4.30 (1.70 to 10.86)	0.002
CRP-level, per mg/L	1.05 (1.01 to 1.09)	0.021
ACPA-positive	6.43 (2.57 to 16.05)	<0.001
Presence of any MRI-detected inflammation	5.07 (1.77 to 14.50)	0.002

Presented are the HRs of multivariable analyses including 142 patients with CSA that underwent MRI of which 27 developed the outcome clinical arthritis. One patient that underwent MRI had missing data on localisation of initial symptoms and was not included in present analysis.

(see online Supplementary Table S3). Hence, MRI-detected inflammation was associated with progression to clinical arthritis, independent of other clinical and serological factors.

Sensitivity analyses on initiation of DMARD treatment

Sensitivity analyses were performed with initiation of DMARD therapy as outcome. Twenty-five out of the 30 patients that developed clinical arthritis were started with DMARD treatment. Repeating the latter multivariable Cox regression analysis (including clinical and serological factors and presence of any MRI-detected inflammation) with DMARD initiation as outcome revealed similar results (data not shown).

Diagnostic value of ACPA and subclinical MRI inflammation

The previous analyses showed that the presence of subclinical MRI inflammation and ACPA were the two strongest and independent factors associated with arthritis development. We continued with determining test characteristics of both factors with arthritis development within the first year as outcome (Table 5). To this end, analyses were restricted to the patients with CSA with 1 year follow-up who had data on ACPA and MRI (n=122). Of these, 21 (17.2%) had developed clinical arthritis within this year. Two patients developed arthritis after the first year had passed; these patients are now categorised in the non-arthritis group.

Test characteristics of ACPA

The sensitivity of ACPA for arthritis development was 57%, indicating that 57% of the patients with CSA that developed arthritis were ACPA-positive and 43% ACPA-negative. Of the patients that developed arthritis 24% were negative for ACPA and RF. The specificity of ACPA was 93%. The positive predictive value (PPV) of ACPA was 63%, indicating that 63% of ACPA-positive patients with CSA have developed clinical arthritis within 1 year (Table 5).

Test characteristics of subclinical MRI inflammation

Subclinical MRI inflammation was present in 81% of the patients that have developed arthritis

Table 5. Test characteristics of ACPA and subclinical MRI-inflammation for arthritis development within 1 year

	Sens (95% CI)	Spec (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	AUC (95% CI)
ACPA in all patients with CSA	57% (48% to 66%)	93% (89% to 98%)	63% (55% to 72%)	91% (86% to 96%)	8.24 (3.69 to 18.44)	0.46 (0.28 to 0.76)	0.75 (0.62 to 0.89)
MRI in all patients with CSA	81% (74% to 88%)	63% (55% to 72%)	31% (23% to 40%)	94% (90% to 98%)	2.21 (1.59 to 3.07)	0.30 (0.12 to 0.74)	0.72 (0.61 to 0.84)
MRI in ACPA-positive patients with CSA	83% (67% to 100%)	43% (21% to 65%)	71% (51% to 92%)	60% (38% to 82%)	1.46 (0.73 to 2.91)	0.39 (0.08 to 1.79)	0.63 (0.36 to 0.90)
MRI in ACPA-negative patients with CSA	78% (70% to 86%)	65% (56% to 74%)	18% (10% to 25%)	97% (93% to 100%)	2.22 (1.42 to 3.46)	0.34 (0.10 to 1.17)	0.71 (0.54 to 0.88)

Presented are the test characteristics for arthritis development within the first year in 122 patients with CSA who had completed 1 year follow-up and had data on ACPA and MRI.

within 1 year (sensitivity=81%). The specificity was 63%. In case of a positive MRI, 31% of the patients progressed to arthritis (PPV) within 1 year and of all persons with a negative MRI at baseline only 6% developed arthritis (100% minus negative predictive value (NPV)) (Table 5). These 6% concerned four patients; three of them developed initial clinical arthritis in a joint that was not depicted on MRI and one developed arthritis 17 weeks after inclusion in joints that were depicted on MRI.

Test characteristics of subclinical MRI inflammation within ACPA-negative and ACPA-positive patients with CSA

Test characteristics for subclinical MRI inflammation were determined within the ACPA-negative and ACPA-positive patients separately to evaluate the diagnostic value in the different sub-groups (Table 5). Although patient numbers became small, especially in the ACPA-positive subgroup, these stratified analyses indicate the value of subclinical MRI inflammation if ACPA results are known. Within the ACPA-positive patients 71% with a positive MRI progressed towards arthritis within 1 year (PPV=71%). Hence, whereas the chance on arthritis in case of ACPA-positivity in the total CSA population was 63%, within the ACPA-positive patients with a positive MRI it was 71%. Of the ACPA-positive patients with a negative MRI 60% did not develop arthritis within 1 year (NPV). Within the ACPA-negative patients with CSA, a negative MRI made the chance on arthritis development very low (3%, 100% minus NPV). Furthermore, the sensitivity of a positive MRI in ACPA-negative patients was 78%. Thus, whereas 43% of the patients that developed arthritis had a negative ACPA test, 78% of these patients were identified by a positive MRI at baseline. Similar results were obtained in ACPA-negative and RF-negative patients (data not shown).

DISCUSSION

This longitudinal study of patients with CSA, with a median follow-up duration of 75 weeks observed that part of the patients with CSA progressed to arthritis. The majority already had subclinical MRI inflammation when presenting with CSA and progressed to arthritis within 4-5 months. These data indicate that the period of CSA and subclinical inflammation is relatively short and encompasses several months.

MRI-detected inflammation is one of the risk factors for arthritis development explored in this study. Thirty-one per cent of patients with CSA with a positive MRI progressed to arthritis within 1 year. Arthritis development within the 1st year was rare (6%) if the baseline MRI was negative.

The association of subclinical MRI inflammation with arthritis development was independent of other factors such as ACPA. Interestingly, the effect sizes in the multivariable analyses of both variables were in the same range. In clinical practice serological results are generally available before imaging tests are ordered. To get a better impression of the additional value of MRI, the analyses on the diagnostic value of MRI were performed in the ACPA-

positive and ACPA-negative subgroups. This revealed that the risk of arthritis development within 1 year was 71% if ACPA-positive patients had a positive MRI. Additionally, 60% of the ACPA-positive patients with a negative MRI did not develop arthritis within 1 year. MRI was valuable in ACPA-negative patients with CSA as the majority (78%) of the ACPA-negative patients that developed arthritis had a positive MRI at baseline.

A strength of this study is that a positive MRI was defined using the prevalence of MRI features in the general population as reference, lowering the risk of false-positive MRI findings. Of the different types of MRI-detected inflammation (synovitis, BME, tenosynovitis), tenosynovitis was most predictive for arthritis development. Previous studies showed that MRI-detected tenosynovitis is frequently present in RA [11] and rarely present in the general population [10].

A limitation is that in the first 77 patients MRI of the feet was made without contrast-enhancement. This may have affected the RAMRIS scores for synovitis on the feet. In this study, we scored MRIs without contrast conservatively, which may have resulted in an underestimation of inflammation on MRIs without contrast enhancement [12]. Another limitation is the median follow-up duration of 75 weeks. It is unsure whether longer follow-up will change our results; nonetheless, we observed that the majority of patients had already progressed in the first months after inclusion.

Further replication studies are needed in other CSA populations before it can be decided if MRI is a useful tool in CSA in daily practice. In this study, MRI was used because an accepted validated scanning and scoring protocol exists [8], it is a minimal operator-dependent procedure and the prevalence of MRI-detected inflammation in the general population is known 10. These issues are not yet solved for ultrasound and further studies are needed to determine whether ultrasound might also be useful in CSA.

Previous studies on patients with symptoms without clinical arthritis evaluated patients with unspecified arthralgia [4,5,13,14]. These studies identified morning stiffness and joint tenderness as predictors for progression to arthritis [4,5]. These clinical factors were not associated with arthritis development in patients with CSA. This is presumably caused by the fact that patients with CSA were selected on the basis of their symptoms and signs. Indeed, the frequency of morning stiffness in CSA was higher than that in unspecified arthralgia [4,5].

The reported risk of developing arthritis within 1 year in autoantibody-positive patients with unspecified arthralgia was 20-34% and up to 41-43% if other risk factors were present [4,5]. Of patients with CSA that were ACPA-positive 63% progressed to arthritis within 1 year, suggesting that the predictive value of ACPA is higher in CSA than in unspecified arthralgia.

The present study is the first exploring the outcome of patients with CSA. We did not aim to derive a prediction rule because the current data set is too small to use part of the data for identification and the other part for validation. In addition, we anticipated that

for accurate prediction more predictors are needed than those entered in our multivariable analysis. Further work is needed to this end.

In conclusion, the phase of CSA without clinically apparent arthritis but with subclinical inflammation encompasses several months. Present data suggest that MRI is diagnostically relevant in this disease phase. With regards to the role of MRI in identifying patients with an increased risk of arthritis, the absolute value of MRI may be higher in ACPA-negative then in ACPA-positive patients with CSA, as ACPA-positive patients with CSA already have a higher prior risk of arthritis development. Importantly, MRI is also useful to rule out imminent arthritis; patients with a clinical suspicion to progress to RA but a negative MRI had a low risk of developing arthritis. Further studies are needed to identify the set of variables that optimally identifies patients with RA in the phase of arthralgia without clinical arthritis and to examine if treatment in this phase is more effective than initiating treatment when clinical arthritis has developed.

SUPPLEMENTARY DATA

Supplementary data are published on the website of the *Annals of the Rheumatic Diseases*.

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Additional File. RAMRIS-based frequencies of synovitis, BME and tenosynovitis per joint/bone, age category and grade of severity; presented are percentages present in symptom-free persons (derived from reference 10)

MCPs									
	Grade 1			Grade 2			Grade 3		
	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years
Synovitis									
MCP-2	0	8	19	0	0	0	0	0	0
MCP-3	0	14	17	0	0	0	0	0	0
MCP-4	0	2	4	0	0	0	0	0	0
MCP-5	0	1	6	0	0	0	0	0	0
BME*									
MCP-2	2	2	4	0	0	0	0	0	0
MCP-3	2	3	6	0	0	0	0	0	0
MCP-4	0	0	0	0	0	0	0	0	0
MCP-5	0	2	0	0	0	0	0	0	0
Tenosynovitis									
Extensor MCP-2	0	0	0	0	0	0	0	0	0
Extensor MCP-3	0	1	0	0	0	0	0	0	0
Extensor MCP-4	0	0	0	0	0	0	0	0	0
Extensor MCP-5	0	0	0	0	0	0	0	0	0
Flexor MCP-2	0	1	6	0	0	0	0	0	0
Flexor MCP-3	0	3	12	0	0	0	0	0	0
Flexor MCP-4	0	3	6	0	0	0	0	0	0
Flexor MCP-5	0	1	2	0	0	0	0	0	0

Wrist									
	Grade 1			Grade 2			Grade 3		
	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years
Synovitis									
Intercarpal-CMC joint	4	16	27	0	0	0	0	0	0
Radio-carpal joint	0	17	35	0	0	0	0	0	0
Distal radio-ulnar joint	0	8	17	0	0	0	0	0	0
BME									
Metacarpal-1 basis	0	3	8	0	0	2	0	0	4
Metacarpal-2 basis	4	1	2	0	0	0	0	0	0
Metacarpal-3 basis	0	0	2	0	0	0	0	0	0
Metacarpal-4 basis	0	0	2	0	0	0	0	0	0
Metacarpal-5 basis	0	0	0	0	0	0	0	0	0
Trapezium	0	0	4	0	0	4	0	0	4
Trapezoid	2	1	6	0	0	0	0	0	0
Capitate	6	3	4	2	0	0	0	0	0
Hamate	0	3	8	0	0	0	0	0	0
Scaphoid	2	7	19	0	0	0	0	0	0
Lunate	6	19	27	0	1	4	0	0	0
Triquetrum	2	6	2	0	0	0	0	0	0
Pisiform	0	0	0	0	0	0	0	0	0
Distal radius	0	0	0	0	0	0	0	0	0
Distal ulna	0	7	8	0	0	0	0	0	0
Tenosynovitis									
I extensor	0	0	0	0	0	2	0	0	0
II extensor	0	0	0	0	0	0	0	0	0
III extensor	0	0	0	0	0	0	0	0	0
IV extensor	0	0	2	0	0	0	0	0	0
V extensor	0	0	0	0	0	0	0	0	0
VI extensor	0	9	12	0	0	0	0	0	0
1 flexor	0	0	0	0	0	0	0	0	0
2 flexor	0	0	0	0	0	0	0	0	0
3 flexor	0	0	0	0	0	0	0	0	0
4 flexor	2	0	2	0	0	0	0	0	0

MTPs									
	Grade 1			Grade 2			Grade 3		
	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years	<40 years	40-59 years	≥60 years
Synovitis									
MTP-1	4	11	13	0	0	2	0	0	0
MTP-2	0	1	0	0	0	0	0	0	0
MTP-3	0	1	0	0	0	0	0	0	0
MTP-4	0	0	0	0	0	0	0	0	0
MTP-5	0	0	4	0	0	0	0	0	0
BME*									
MTP-1	10	12	23	0	1	8	0	0	0
MTP-2	2	0	0	0	1	0	0	0	0
MTP-3	0	1	0	0	0	0	0	1	0
MTP-4	0	1	0	0	0	0	0	0	0
MTP-5	0	1	4	0	0	0	0	0	0

Presented are the percentages out of the total number of persons in each age category: 51 symptom-free persons were included in the category <40 years, 90 persons in the category from 40-59 years and 52 persons in the category ≥60 years. These tables are used for present study of patients with CSA to derive age, MRI-feature and location specific reference values for a 'positive MRI'. The locations that were inflamed in ≥5% of individuals are highlighted in dark. Joints/bones in the CSA-patients with scores as presented in in the light areas are categorized as positive for MRI-detected inflammation and the joints/bones with scores as presented the dark areas as negative.
* BME is scored in the proximal and distal MCP and MTP bones separately. The scores of the 2 bones are summed into a 1 score, therefore the range is 0-6 in the MCP and MTP joints. Five bones scored a grade 2 in MTP-1 this consists of 4 persons with a grade 1 in both the proximal and the distal bone and 1 person had a score of 2 in the proximal bone of MTP-1

Clinical expertise and its accuracy in differentiating arthralgia patients at risk for rheumatoid arthritis from other patients presenting with joint symptoms

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Sir, Within RA, early initiation of treatment is associated with a higher chance of achieving DMARD-free sustained remission [1–3]. The absolute number of RA patients achieving this beneficial condition is still low. It is hypothesised that treatment initiation in the early phase of symptoms, without clinically detectable arthritis, will be more effective in disease modulation and will reduce the persistent nature of the disease [4]. Trials are needed to study this hypothesis. For this purpose, we need to identify patients in a symptomatic phase before synovitis is clinically apparent.

The clinical presentation characterizing RA in the early symptomatic phase of this disease is unknown. A method for differentiating patients with arthralgia at risk of RA from other patients with joint symptoms is to use clinical expertise as a starting point. Rheumatologists see many patients presenting with arthralgia, but without clinically apparent arthritis. These patients fall into three categories: patients with a clear diagnosis for their arthralgia; patients without a clear diagnosis but not considered at risk for RA according to their rheumatologists (unexplained arthralgia); and patients with clinically suspect arthralgia (CSA) - based on their clinical presentation, rheumatologists suspect these patients will progress to RA [5]. This study explored the diagnostic accuracy of the clinical expertise of rheumatologists.

Between April 2012 and December 2013, 145 newly referred patients to the rheumatologic outpatient clinic in Leiden (the Netherlands) were identified as having CSA. In the same period, 1791 newly referred patients had unexplained arthralgia according to the local registry that records the diagnosis at first visit for financial purposes. Patients with arthralgia were considered to have reached the outcome when they had developed clinical arthritis within 1 year after first presentation with arthralgia and fulfilled the 1987 classification criteria for RA. For CSA patients, this was determined within the CSA cohort [5]. For the unexplained arthralgia patients, the outcome was determined by investigating which of the 1,791 patients were included in the Leiden Early Arthritis Clinic (EAC) cohort within 1 year after first presentation and also fulfilled the 1987 criteria [6]. To ensure that no converters were missed in the unexplained arthralgia group, all final diagnoses according to the mentioned registry were checked, and all files were checked for patients in whom the diagnoses remained unexplained but who had more than four visits at the outpatient clinic. These additional ways to search did not yield any additional patient with 1987 RA. All patients included in the CSA and EAC cohorts gave informed consent, and approval for these cohorts was obtained from the Medical Ethics Committee of the Leiden University Medical Center. The approval included collection of clinical and serological data and the use of these data for analyses, including this analysis.

At the 1-year follow-up, 16 of the patients identified as CSA patients had progressed to arthritis and fulfilled the 1987 criteria for RA within 1 year (11%). Likewise, 4 of the 1,791 unexplained arthralgia patients were included in the EAC and fulfilled the 1987 criteria (0.2%). The odds ratio was 55 (95% CI=18-168, $p<0.001$), the sensitivity of the clinical

expertise was 80%, the specificity was 93% and the accuracy was 93%. The four RA patients who had initially presented with arthralgia and who were not identified as having CSA had the following presentations: one patient had arthralgia with inflammatory symptoms (morning stiffness, most severe symptoms in early morning), but the rheumatologist did not label the patient as having CSA; one patient had inflammatory symptoms and psoriasis and was suspected of progressing towards psoriatic arthritis instead of RA, and therefore was not labelled as having CSA; in one person the symptoms were attributed to a recent Hepatitis B vaccination; and one person had no inflammatory symptoms or signs at all.

The performance of autoantibody testing in the diagnostic process of arthritis and RA in first-line care is not promoted by the Dutch guideline for general practitioners [7]. More locally in the Leiden area, general practitioners are even discouraged from performing autoantibody tests before referral, and they are encouraged to refer promptly. In line with this, the large majority of patients were referred without results for ACPA or RF. Hence, the diagnosis of having CSA was essentially based on symptoms and signs. We realise that this health care system is differently organised than that in other parts in Europe. Therefore, our setting provides a unique opportunity for exploring the accuracy of clinical expertise based on symptoms and signs only. After the first visit to the outpatient clinic, autoantibody testing was carried out. Three of the four patients who were not identified as having CSA based on their clinical presentation and who did develop RA were ACPA-positive. This illustrates that the evaluation of the presence or absence of CSA was not driven by results of autoantibody status. We do not have data on ACPA-status for the patients with unexplained arthralgia who did not progress to RA. Therefore, this study does not allow us to identify the predictive value of ACPA testing in patients who are clinically not suspected of progressing towards RA.

In conclusion, the present data revealed the value of the rheumatologists' expertise in differentiating arthralgia patients based on clinical presentation (history, symptoms, signs) only. A potential disadvantage is its subjectivity. Therefore, a current EULAR taskforce is deriving criteria for CSA, using a consensus-based approach [8].

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Definition of arthralgia suspicious for progression to rheumatoid arthritis; results of a EULAR taskforce

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[Submitted]

ABSTRACT

Background

During the transition to rheumatoid arthritis (RA) many patients pass through a phase characterised by the presence of symptoms without clinically apparent synovitis. These symptoms are not well-characterised. This taskforce aimed to define the clinical characteristics of patients with arthralgia who are considered at risk for RA by experts based on their clinical experience.

Methods

The taskforce consisted of 18 rheumatologists, 2 patients, 3 health professionals and 1 fellow. The process had three phases. In phase-1, a list of parameters considered characteristic for clinically suspect arthralgia (CSA) was derived; the most important parameters were selected by a three-phased Delphi-approach. In phase-2, the experts evaluated 50 existing patients on paper, classified them as CSA/no-CSA and indicated their level of confidence. A provisional set of parameters was derived. This was studied for validation in phase-3, where all rheumatologists collected patients with and without CSA from their outpatient clinics.

Results

The comprehensive list consisted of 55 parameters, of which 16 were considered most important. A multivariable model based on the data from phase-2 identified 7 relevant parameters: symptom duration <1-year, symptoms of MCP-joints, morning stiffness duration ≥60 minutes, most severe symptoms in early morning, first-degree relative with RA, difficulty with making a fist, positive squeeze-test MCP-joints. In phase-3, the combination of these parameters was accurate in identifying arthralgia patients who were considered at risk of developing RA (AUC=0.92, 95%CI=0.87-0.96). Test characteristics for different cut-off points were determined.

Conclusion

A set of clinical characteristics for patients with arthralgia who are at risk of progression to RA was established.

INTRODUCTION

The development of rheumatoid arthritis (RA) is a multistep process. A European League Against Rheumatism (EULAR) study group differentiated the following phases: (1) presence of genetic and environmental risk factors for RA, (2) systemic autoimmunity associated with RA, (3) symptoms without clinical arthritis, (4) unclassified arthritis and finally (5) RA [1]. The symptomatic phase preceding clinical arthritis is the first opportunity to clinically recognise patients who are at risk for progression to RA. In contrast to the other phases that have been studied extensively, this phase is less well studied. Whilst a few studies reported on symptoms experienced by patients in this phase and on their impact on daily life [2–4], clinical characteristics that are specific for this phase have not yet been identified by a consensus-based approach [1,5,6]. This situation hampers the conduct of studies and clinical trials in this phase of the disease. It has been shown that early initiation of disease modifying anti-rheumatic drug (DMARD) treatment in RA is more effective in modulating the erosive and persisting nature of RA compared to delayed initiation of DMARD treatment [7–9]. Hence interventions in the initial clinical phase of the disease, which precedes the onset of clinical arthritis, may be more effective in reducing the risk of disease persistence and the development of damage [10]. However, studies to address this require the inclusion of homogeneous sets of patients.

Clinical expertise, which includes pattern recognition, guides decisions in daily practice and has also been used as reference for the development of several tools or criteria in the field of rheumatology [11–14]. Patients with Clinically Suspect Arthralgia (CSA) have articular symptoms without signs of arthritis and are considered to be at increased risk for progression to RA [15]. Hence, the identification of the presence of CSA is based on clinical expertise. Recent data revealed that patients with CSA constitute only a small percentage of all patients with arthralgia who visit the rheumatology outpatient clinic for the first time (~7%), and that a proportion of patients with CSA did indeed progress to RA during follow-up (~20%) [16]. It was also suggested that clinical experience was accurate to distinguish patients with arthralgia at risk of RA from other arthralgia patients (OR 55). In particular, only a minority of patients who presented with arthralgia and subsequently developed RA were not recognised by the rheumatologist [17].

Although the concept of CSA is appropriate for use in clinical practice, a drawback is its subjectivity, which may result from differences in practice and experience. Therefore the phenotype of CSA needs to be defined. This taskforce aimed to identify a combination of clinical features that best characterise patients with arthralgia who are at risk of RA according to an expert multidisciplinary group of European rheumatologists, other health professionals and patients. This approach was similar to that which led to the definition of inflammatory back pain, a definition which was subsequently integrated in the ASAS classification criteria [18,19]. The taskforce intended to derive a set of clinical parameters to enable the inclusion of homogeneous sets of patients in subsequent studies. It was considered inappropriate to use the

phrase 'classification criteria' for the product as, basically, classification concerns testing the presence or absence of a disease. CSA is not in itself a disease, but a combination of symptoms and signs. It was anticipated that clinical characteristics alone are insufficient predictive for RA, that a combination of clinical and other factors (e.g. autoantibodies, imaging results) are necessary to identify patients with imminent RA, and that the derived clinical definition can later become part of criteria for imminent RA. Thus in sum, the present taskforce aimed to define arthralgia at risk for RA.

METHODS

Expert committee

The expert committee comprised 18 rheumatologists, one methodologist (RL, who was also one of the rheumatologists), two nurse specialists, one physiotherapist, two patients and one research fellow, originating from 15 European countries. The target populations are rheumatologists and health professionals working in secondary care.

Three-phased process

The process consisted of three phases and two meetings. Expert opinion was the reference. Per phase consensus was obtained before proceeding to the next phase.

Phase-1

Phase-1 aimed to develop a comprehensive list of clinical parameters (both symptoms at history taking and signs at physical examination) that were considered by the experts to be relevant to distinguish arthralgia that precedes RA from other types of arthralgia. A modified Delphi approach was used. First, all taskforce members were asked to indicate all symptoms and signs that they considered potentially relevant. All parameters mentioned to be relevant by at least two experts or by the patients (based on personal experience) were added to create a comprehensive list. In the next three quantitative rounds the participants selected the parameters they considered most relevant by weighing. After each round, the list of parameters was modified based on the results; parameters on which consensus was reached (either relevant or irrelevant) were not evaluated in the next round. The group response of the previous round and the modified list were presented to the group before they voted in the next round.

Phase-2

Phase-2 aimed to develop a provisional set of clinical parameters describing CSA. The experts reviewed clinical data from 50 patients who had previously presented with arthralgia but without clinically detectable arthritis to the rheumatology outpatient clinic of the Leiden University Medical Centre (the Netherlands). Of these, 26 were considered to have CSA by the treating rheumatologist [15]; the prevalence of CSA in this patient set was thus artificial and much higher than in a general rheumatology outpatient clinic. The experts were blinded for

grouping by the treating rheumatologists. Clinical data relating to the parameters selected in Phase-1 were presented to the experts as being present or absent in these 'paper patients'. The experts were asked to classify each patient as having CSA or no-CSA and to provide the level of confidence with their classification on a numerical rating scale from 0 (not confident) to 10 (very confident).

Two approaches were used to analyse the data from Phase-2. First, to gain insight into the degree of equivalence of the expert classifications, the frequencies of the classifications were plotted against the level of confidence of each classification per patient, as described previously [19]. Individual histograms represented all experts' judgments on individual patient and were evaluated independently by three reviewers (AvdHvM, RL, HvS); each reviewer decided whether the experts agreed on the classification as 'CSA', 'no-CSA' or 'unclassifiable'. If all reviewers had the same judgment the patient was categorised accordingly. Otherwise, agreement between the reviewers was reached on how to categorise a patient. The parameters selected in phase-1 were compared for the patients in the three groups (CSA, no-CSA and unclassifiable). Then, to statistically identify the parameters that best discriminated between CSA and no-CSA, a multilevel model was used with one level being the expert and the other level being the patient; this analysis which was done on 900 judgments about CSA included the data of all 50 patients, each classified by 18 rheumatologists. This mixed effects model with crossed random effects was applied with the weighted CSA classification as outcome and the clinical parameters as independent variables. This model was used to take into account that each expert assessed the same 50 patients. Crossed random effects were included as the symptoms are nested in the combination of expert and patient and thus the residuals of the two levels are still correlated, even after taking the two levels of the analysis into account [20,21]. Clinical parameters with a p-value ≤0.05 in univariable analyses were included in multivariable analysis. The parameters with a positive coefficient in the multivariable analysis were combined to a provisional set of parameters describing CSA. These data were presented at the first meeting.

Phase-3

Phase-3 aimed to validate the provisional set of parameters in the outpatient clinics of the participating rheumatologists. They were asked to select newly referred patients without a defined time limit of symptoms and without arthritis but with arthralgia who they considered to have an increased risk of RA based on history taking and physical examination (patients with CSA) and patients who had no evident diagnosis or explanation for the arthralgia at first visit but were not considered at risk for RA (no-CSA). Patients who at presentation had evident diagnoses, such as fibromyalgia or osteoarthritis, were not included in the no-CSA group. In addition, the participants were encouraged to base the decision of CSA on the clinical presentation only and not on results of additional investigations. Due to differences in health care settings, some rheumatologists had access to the result(s) of laboratory or imaging investigation(s) at first presentation for the majority of their patients. The presence or absence

of additional test results at the time of identification of CSA or no-CSA was recorded. The provisional set of parameters derived from phase-2 was tested using multivariable logistic regression analyses in the identified CSA and no-CSA patients. Thus again clinical expertise was the reference. The performance of the combination of parameters was assessed using the area under the receiver operating characteristic curve (AUC). Sensitivity and specificity were determined for different cut-off points. The data from this phase were discussed during the second meeting. The final set of parameters was established by voting.

RESULTS

Phase-1 – Identifying relevant parameters for CSA

First, all experts identified as many parameters as possible that they considered relevant when evaluating whether arthralgia patients did or did not have CSA. The total list consisted of 55 parameters (Supplementary Table S1) and included both parameters that were considered to increase and decrease the likelihood of CSA. By selecting and weighing in three rounds, the number of parameters on the list was reduced to 16 (Table 1). Consensus was reached to proceed with these 16 parameters to phase-2.

Phase-2 – Development of provisional set of parameters describing CSA

First, in order to get an overview of the data, each of the 50 patients were classified as having CSA, no-CSA or being unclassifiable based on their individual histograms which represented the classifications of all experts. Seventeen patients were unequivocally classified as no-CSA, 14 as CSA and 19 patients were considered unclassifiable (examples of the histograms are presented in Supplementary Figure S1). Table 1 presents the frequencies of the clinical parameters for the groups of patients identified as no-CSA, unclassifiable and CSA.

Then, using data from all 50 patients, a multilevel model with weighted CSA classification as outcome was used to select the parameters that best discriminated between CSA and no-CSA. Results of univariable and multivariable analyses are presented in Supplementary Table S2. The following 7 variables were presented during the first meeting as a provisional set of parameters describing CSA: joint symptoms of recent-onset (duration <1 year), symptoms located in MCP-joints, symmetric symptoms or signs (bilateral in same joint region), duration of morning stiffness ≥60 minutes, most severe symptoms present in the early morning, difficulty with making a fist and positive squeeze-test of MCP-joints. At the meeting, it was suggested to remove the item symmetry from the multivariable analysis (because of $p>0.05$ in univariable analysis) and to force MTP-involvement and a positive family history in the analysis (as these items were judged as very relevant by many experts). The results are presented in Supplementary Table S3. Thereafter, consensus was reached on the following 7 parameters to characterise arthralgia that is clinically suspect for progression to RA: joint symptoms of recent-onset (duration <1 year), symptoms located in MCP-joints, duration of morning stiffness ≥60 minutes, most severe symptoms present in the early

Table 1. Parameters that were selected in phase-1, and frequencies of these parameters in the patients that in phase-2 were categorised as CSA, no-CSA or were considered unclassifiable

	No-CSA (n=17)	Unclassifiable (n=19)	CSA (n=14)
History taking			
Joint symptoms of recent-onset (duration <1 year)	41%	74%	92%
4-10 joints with symptoms	47%	57%	21%
Symptoms in MCP-joints	35%	63%	93%
Symptoms in MTP-joints	35%	53%	57%
Symptoms in several small joint regions (MCP, wrists, PIP, MTP-joints)	35%	68%	93%
Symmetric symptoms or signs (bilateral in same joint region)	77%	58%	100%
Duration of morning stiffness ≥60 minutes	6%	37%	71%
Most severe symptoms in the early morning	27%	69%	90%
Improvement of symptoms during the day	15%	36%	90%
Increasing number of joints with symptoms over time	70%	71%	90%
Patient-experience of swelling of small hand joints	31%	47%	77%
Presence of a first-degree relative with RA	7%	33%	36%
Physical examination			
Difficulty with making a fist	8%	31%	43%
Local tenderness involved joints at physical examination	63%	84%	86%
Positive squeeze-test of MCP-joints	14%	26%	69%
Positive squeeze-test of MTP-joints	22%	21%	39%

Data on symptoms of recent-onset was missing in 1 patient, on most severe symptoms in early morning in 6 patients, on improvement of symptoms during the day in 8 patients, on increasing number of joints with symptoms over time in 11 patients, on patient-experience of swelling in 2 patients, on difficulty with making a fist, presence of a first-degree relative with RA, local tenderness of joints, squeeze-test of MCP- and MTP-joints in 4 patients.

morning, presence of a first-degree relative with RA, difficulty with making a fist and positive squeeze-test of MCP-joints (Box 1).

Phase-3 – Validation

In total 322 patients with arthralgia were identified in the different centres (Supplementary Table S4), 142 patients with CSA and 180 arthralgia patients without CSA. Of them, 78 and 61 respectively were identified based on clinical information only (i.e. without data relating to additional investigations); these 139 patients were used in the main analysis. When weighing the parameters based on the B coefficient of the logistic regression analysis after rounding the coefficients to whole points, the combination of 7 parameters performed well to explain the clinical expertise (AUC 0.93, 95%CI 0.89-0.97). When using all variables unweighted, the combination of 7 parameters performed equally well in identifying arthralgia patients who were considered to be at risk of RA by the experts (AUC 0.92, 95%CI 0.87-0.96) (Supplementary Table S5). The experts agreed that unweighted parameters were more convenient. When analysing all 322 patients, similar AUCs were obtained (Supplementary

Table S6).

The sensitivities and specificities belonging to the number of positive parameters are presented in Table 2. A sensitivity >90% was obtained in the presence of ≥3 parameters and a specificity >90% in the presence of ≥4 parameters. All taskforce members unanimously agreed that arthralgia that is suspected for progression to RA is defined by the seven parameters presented in Box 1 and that these parameters are to be used in patients with arthralgia but not clinical arthritis in whom there is not a better explanation for the arthralgia.

DISCUSSION

The development of RA is a multi-step process. In this project we defined the combination of symptoms and signs that characterise patients at risk of developing RA. In clinical practice, rheumatologists identify patients with CSA based on their expertise. The presence of CSA may trigger rheumatologists to monitor patients closely and/or to undertake specific laboratory testing or imaging. For daily rheumatologic practice the concept of CSA has been shown to be adequate to differentiate patients with arthralgia [16,17], but it is subjective and this results

Box 1. EULAR defined characteristics describing arthralgia at risk for RA

These parameters are to be used in patients with arthralgia without clinical arthritis and
without other diagnosis or other explanation for the arthralgia.

History taking:
- Joint symptoms of recent-onset (duration <1 year)
- Symptoms located in MCP-joints
- Duration of morning stiffness ≥60 minutes
- Most severe symptoms present in the early morning
- Presence of a first-degree relative with RA

Physical examination:
- Difficulty with making a fist
- Positive squeeze-test of MCP-joints

Table 2. Sensitivities and specificities for the presence of arthralgia at risk of RA with the clinical expertise on CSA as reference

Number of parameters present	Sensitivity	Specificity
≥ 1	100.0%	14.1%
≥ 2	98.4%	53.8%
≥ 3	90.2%	74.4%
≥ 4	70.5%	93.6%
≥ 5	32.8%	100.0%
≥ 6	16.4%	100.0%
≥ 7	1.6%	100.0%

in heterogeneity. For scientific studies homogeneous sets of patients are required. Therefore, we aimed to capture clinical expertise and represent it in a set of defined clinical parameters. The process incorporated three phases and two meetings, and the product was obtained by a data-driven and consensus-driven approach. Unanimous agreement was obtained on seven parameters reflecting the aggregated expertise of rheumatologists, health care professionals and patients from fifteen European countries.

This taskforce was able to successfully identify and collate a homogenous and measurable set of clinical parameters of CSA based on clinical expertise of rheumatologic experts for use in future studies. Further longitudinal studies are required to assess if this definition reduces the number of arthralgia patients that need additional testing, and to determine the predictive accuracy of these clinical parameters for the development of RA, both when used alone and in combination with the results of additional investigations. Thus, the result of this taskforce should serve as the basis for the next step, which is the initiation of longitudinal data-driven projects, which ultimately results in the development of criteria for imminent RA. Most likely such criteria will include both clinical and investigation based parameters (such as laboratory and imaging results).

Because a clinical definition alone is unlikely to be sufficiently accurate to identify RA patients in a symptomatic pre-arthritis phase, and because CSA is not a disease but the description of a phenotype, it was decided that the product of this taskforce should not be referred to as 'classification criteria' but as a 'definition'. Furthermore, while the physicians in the taskforce argued that the word 'patient' may have an unwarranted connotation, the patient representatives in the task force justified the use of the term 'patient' by pointing to the fact that these individuals had presented with pain and other symptoms and had been referred to secondary care.

The parameters characterising arthralgia at risk of RA may serve as the basis for observational studies and intervention trials performed in the symptomatic pre-arthritis phase. Depending on the study a more sensitive or more specific definition may be preferred. A high sensitivity may be preferred if the clinical criteria are used as first inclusion criterion, as in this situation the number of CSA patients that are missed by the criteria should be low. Subsequently, additional tests can be applied to ensure sufficient specificity. If in contrast, patients are mainly selected based on clinical characteristics, a higher specificity may be preferred to prevent false-positives. Given this, the taskforce deliberately avoided a single cut-off point to define arthralgia at risk of RA, but provided the test characteristics of a spectrum of cut-off points. A high sensitivity (>90%) is obtained if ≥3 out of the 7 parameters are present; a high specificity (>90%) requires the presence of ≥4 of the 7 parameters.

The clinical variables were considered to distinguish arthralgia patients who are at risk of RA from patients with other types of (not specified) arthralgia. Patients that at first presentation clearly had other diagnoses, such as fibromyalgia or osteoarthritis, were not

included in the control groups of phases 2 and 3. This is in line with clinical practice, as there is no diagnostic dilemma in the patients with evident diagnoses. Similar to the 2010 ACR/EULAR classification criteria for RA that should not be applied to arthritis patients with diagnoses other than RA [14], the present set of parameters is reserved for patients with arthralgia with no definitive diagnosis but a clinical suspicion of RA.

The definition was derived for use in secondary care. Because of this target population, the taskforce was composed largely of rheumatologists and their expertise was used as a reference. General practitioners were not involved. The taskforce discussed whether our present product may be useful as a referral tool for general practitioners, as has been done by others [22]. Whilst the taskforce was of the opinion that the present set of parameters might also be valuable to identify patients with arthralgia at risk of RA in primary care, it was agreed that the applicability of the present definition in the primary care setting would need to be assessed through future research in primary care.

It was acknowledged that there may be some redundancy in the seven parameters expressing risk for RA. Further prospective studies will be required to elucidate if one of the parameters can be omitted without losing discriminative ability.

A limitation of our approach is that the experts who developed the list of relevant parameters in Phase-1 and scored the patients in Phase-2 also identified patients for the validation phase. It is possible that the discussions that were held and the data from the first two phases influenced their clinical expertise while selecting patients with CSA and arthralgia patients without CSA. However many experts also involved other colleagues to select patients with CSA from their clinics and these colleagues were not involved in the first two phases of the project.

Differences in health care settings affect the ability to identify patients in a symptomatic phase prior to presenting with clinically apparent arthritis. E.g., between centres and countries there are differences in the possibilities for early access. Some of the differences between health care settings were incorporated by inviting experts from different centres and different countries and by using a consensus-based approach. There were also differences in the extent to which additional investigations were performed prior to the first clinical evaluation in speciality care. As the aim of the taskforce was to provide a clinical definition, and as knowledge of the results of additional investigations may influence the selection of patients in phase 3, patients in whom knowledge of additional investigations were known at first presentation were initially excluded from analyses. This ensured that patients were exclusively identified on the clinical presentation. However, a sub-analysis including also the other patients did not give different results, revealing robustness of the data.

The taskforce had discussed if the individual parameters needed to be defined. Consensus was derived that this project was not aiming at what definition of a specific domain was best, but rather what domains contribute most to the 'phenotype' of CSA, given

all the restrictions.

In conclusion, a set of clinical characteristics describing arthralgia at risk of RA was established. The combination of these parameters accurately reflected expert opinion about CSA. Test characteristics were determined for different cut-off points. For a sensitive definition, arthralgia at risk of RA can be defined by the presence of ≥3 parameters and the presence of ≥4 parameters yielded a high specificity. Longitudinal studies are required to determine the predictive accuracy of these clinical parameters alone and when combined with the results of additional investigations, such as laboratory testing or imaging.

SUPPLEMENTARY DATA

Supplementary data are available from the author upon request.

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Part II

Genetic factors and disease outcome in rheumatoid arthritis

Predicting the severity of joint damage in rheumatoid arthritis; the contribution of genetic factors

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8

ABSTRACT

Introduction

The severity of radiologic progression is variable between rheumatoid arthritis (RA) patients. Recently, several genetic severity variants have been identified and were replicated, these belong to 12 loci. This study determined the contribution of the identified genetic factors to the explained variance in radiologic progression and whether genetic factors, in addition to traditional risk factors, improve the accuracy of predicting the severity of radiologic progression.

Methods

426 early RA patients with yearly radiologic follow-up were studied. The main outcome measure was the progression in Sharp-van der Heijde score (SHS) over 6 years, assessed as continuous outcome or categorised in no/little, moderate or severe progression. Assessed were improved fit of a linear mixed model analysis on serial radiographs, R^2 using linear regression analyses, C-statistic and the net proportion of patients that was additionally correctly classified when adding genetic risk factors to a model consisting of traditional risk factors.

Results

The genetic factors together explained 12–18%. When added to a model including traditional factors and treatment effects, the genetic factors additionally explained 3–7% of the variance (p-value R^2_{change}=0.056). The percentage of patients that was correctly classified increased from 56% to 62%; the net proportion of correct reclassifications 6% (95% CI 3 to 10%). The C-statistic increased from 0.78 to 0.82. Sensitivity analyses using imputation of missing radiographs yielded comparable results.

Conclusion

Genetic risk factors together explained 12–18% of the variance in radiologic progression. Adding genetic factors improved the predictive accuracy, but 38% of the patients were still incorrectly classified, limiting the value for use in clinical practice.

INTRODUCTION

The severity of rheumatoid arthritis (RA) is commonly expressed by the extent of damage of hand and feet joints. Joint damage can be measured objectively with validated scoring methods and is associated with long-term functional disability [1]. The severity is highly variable between patients; many patients show mild progression and few severe progression. The processes underlying these differences are partly understood. The observation that the heritability of radiologic progression is 45-58% [2] underlined the notion that genetic factors play a role. Presently, several genetic risk factors for radiologic progression have been identified and replicated. Some of these variants were also associated with differences in mRNA or protein expression [3–6]. Here, we aimed to explore the relevance of currently known genetic risk factors with regards to (1) explaining the interindividual variance in radiologic progression and (2) improving the accuracy of predicting radiologic progression for individual patients.

Known traditional risk factors explain about one-third of the variance in joint damage after 5 years of disease; the majority of these risk factors were related to patient characteristics (age, gender), inflammation (acute phase reactants, swollen joint counts) and the presence of auto-antibodies [7]. The contribution of the genetic risk factors to the explained variance has not been explored.

Prediction of RA severity on the level of individual patients is not yet accurate. Several matrices to predict rapid radiologic progression have been derived, consisting of three or four risk factors. Most of these matrices are not validated in the general RA population, and failed to correctly classify ~50% of patients. In particular, the patients who developed progressive disease were not recognized [8–13]. Consequently, the value of these matrices for clinical practice is still limited. Whether the addition of genetic factors improves prediction is unknown.

This study examined the variance in joint damage progression explained by recently identified genetic risk factors and their value in improving the prediction of the severity of joint damage progression. We assessed traditional performance measures of prediction models and the net proportion of RA patients that is additionally correctly classified when adding risk factors to a prediction model consisting of known risk factors.

PATIENTS AND METHODS

Patients

Between 1993 and 2006, 600 RA patients (1987-ACR-criteria) were included in the Leiden Early Arthritis Clinic (EAC) [7]. Inclusion in the EAC took place when arthritis was confirmed at physical examination and symptom duration was less than 2 years. At first visit, patients and rheumatologists filled questionnaires, 66-swollen and 68-tender joint counts were performed (66-SJC and 68-TJC [14]), and blood samples were taken. Patients were followed yearly. The initial treatment strategy differed for different inclusion periods: patients included in 1993–

1995 were initially treated with NSAIDs, patients included in 1996–1998 were initially treated with hydroxychloroquine or sulphasalazine, and patients included since 1999 were promptly treated with methotrexate. The severity of radiologic progression differed for these three treatment groups; therefore, treatment effects were incorporated in the analyses. The traditional risk factors studied were age, gender, symptom duration at first visit, localisation initial joint symptoms, 66-SJC, presence of anti-citrullinated peptide antibodies (ACPA), presence of rheumatoid factor (RF) and erythrocyte sedimentation rate (ESR).

Selection of genetic risk factors and genotyping

We selected single nucleotide polymorphisms (SNPs) using the following criteria: the SNP was studied in relation to the severity of radiologic progression in several cohorts and the association was independently replicated or found significant in a meta-analysis including all published data. Based on these criteria, we came to a selection of genetic variants that is presented in table 1. Notably, rs4810485 in *CD40* and rs7607479 in *SPAG16* were identified as risk factors for radiologic progression only in ACPA-positive RA. Genotypings in the EAC

Table 1. Genetic variants studied and the R2 of each variant for radiologic progression over six years.

Genetic variant(risk allele)	Located in/nearby gene(s)	Chr.	MAF*	Tested Model*	R^2 $\Delta SHS_{0\text{-}6years}$ (%) in RA (n=239)	R^2 $\Delta SHS_{0\text{-}6years}$ (%) in ACPA-pos RA (n=144)
SE [29]	*HLA-DRB1*	6	0.39	add	4.0	<0.01
rs4810485 (T) [15]	*CD40*	20	0.24	rec	0.1	<0.01
rs7667746 (G) [16]	*IL-15*	4	0.33	rec	2.6	3.9
rs7665842 (G) [16]	*IL-15*	4	0.40	rec	2.7	3.7
rs4371699 (A) [16]	*IL-15*	4	0.19	rec	0.3	1.0
rs6821171 (C) [16]	*IL-15*	4	0.29	rec	0.1	1.4
rs1896368 (G) [4]	*DKK-1*	10	0.47	add	0.3	1.1
rs1896367 (A) [4]	*DKK-1*	10	0.41	add	0.4	0.7
rs1528873 (A) [4]	*DKK-1*	10	0.47	add	2.1	3.0
rs2104286 (C) [18]	*IL2RA*	10	0.24	add	0.3	<0.1
rs8192916 (A) [3]	*GRZB*	14	0.42	rec	0.8	1.4
rs1119132 (A) [17]	*IL-4R*	16	0.13	rec	0.5	1.1
rs7607479 (C) [6]	*SPAG16*	2	0.33	add	0.6	2.5
rs26232 (T) [19]	*C5orf30*	5	0.29	add	0.3	<0.1
rs11908352 (A) [5]	*MMP-9*	20	0.21	add	4.7	1.3
rs451066 (A) [5]	rs1465788	14	0.20	add	1.1	0.2
rs1485305 (T) [30]	*OPG*	8	0.44	add	1.4	0.6

The presented R^2s were based on univariable analyses for each individual risk factor. *The MAFs and tested models are presented as reported in the previous studies. MAF=minor allele frequency; R^2= proportion of explained variance; $\Delta SHS_{0\text{-}6years}$= progression in Sharp-van der Heijde score over six years; add=additive; rec=recessive; SE=shared epitope.

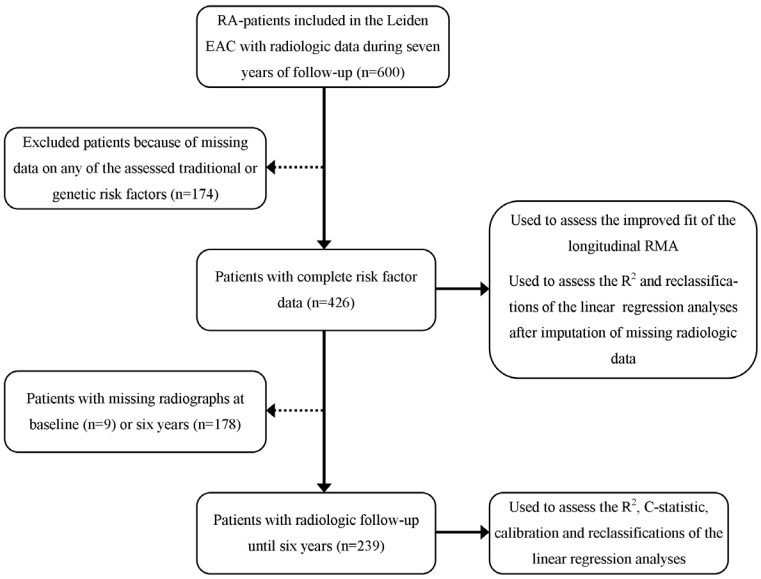


Figure 1. Flow chart of patient selection.
Baseline characteristics of the included (n=426) and excluded patients (n=174) were not different (data not shown). The patients with follow-up until six years (n=239) were younger compared to the patients without complete follow-up until six years (n=187) (mean (SD) 53.9 (14.5) versus 60.0 (15.7) years, p<0.001), had a higher 66-SJC (median (IQR) 9 (5–16) versus 8 (3–13), p=0.009) at baseline and were more frequent ACPA-positive (60.3% vs 44.4%, p=0.001). RMA=repeated measurement analysis; R^2=proportion of explained variance.

were done with allele-specific kinetic PCR analysis [15], Illumina Golden Gate platform [3,4,16,17], Illumina Immunochip [5,18], Sequenom iPLEX [6] and LightSnp (Roche) [19]. Quality control of genotyping was performed as described previously [3–6,15–19]. 426 patients had complete data on all evaluated traditional and genetic risk factors (figure 1).

Radiologic outcome

X-rays were taken at baseline and with yearly intervals. Totally, over 7 years, 2680 X-ray sets of hands and feet of 426 patients were made and scored by one experienced reader using Sharp-van der Heijde scores (SHSs) blinded to any clinical or genetic data (intraclass correlation coefficient 0.91). The numbers of patients with radiologic data at baseline and over 4, 5, 6 and 7 years were, respectively, 321, 286, 239 and 206. The main outcome measure in this study was radiologic progression in the first 6 years after inclusion ($\Delta SHS_{0-6\ years}=SHS_{6\ years}-SHS_{0\ years}$). Although radiologic data was known up till 7 years, the progression over 6 years was chosen as main outcome as fewer patients completed 7 years of follow-up. For some accuracy measures the continuous outcome was categorised in three groups of progression over 6 years: $\Delta SHS_{0-6\ years}$ ≤6, 7–30 and >30 units, indicating no/little, moderate and severe radiologic progression (figure 3A). The first cut-off was chosen as progression of ≤1 SHS-unit per year is minimal; the latter cut-off was chosen because rapid radiologic progression is generally defined as an increase of 5 SHS-units per year [8–10]. In all analyses, the difference in

SHS was log10-transformed to approximate a normal distribution.

Analyses

The performance of prediction models can be evaluated using different aspects, see table 2 [20,21]. Inherent to the method of determining these aspects, the radiologic progression rate over 6 years was studied as a continuous or categorised outcome.

Improved fit - First, a linear mixed model analysis was used with serial log10-transformed SHS over 6 years as response variable and time and risk factors as variables.

Table 2. Different measures to evaluate the performance of prediction models; inherent to the statistical method used, progression over six years was assessed as a continuous or categorical outcome variable.

Aspect and measure	Characteristics	Used model and radiologic outcome
Overall performance: 'goodness-of-fit' of model, quantification of how close predictions are to the observed outcome; captures both aspects of calibration and discrimination		
Improved fit	Improved fit of model after adding additional variables to the model (%). Measured as relative increase in Nagelkerke R^2 (modified version of Cox and Snell's pseudo R2) 23.	Linear mixed model analysis with yearly SHS scored X-rays over six years as outcome. Patients with missing radiographs at a certain time point were included.
R^2	Variance in outcome explained by the included variables. The explained variance can be corrected for the number of variables in the model (adjusted R^2) (%).	Linear regression analysis with radiologic progression between baseline and six years ($\Delta SHS_{0\text{-}6years}$) on a continuous scale as outcome. Analyses are done on patients with complete data (n=239) and on all patients (n=426) when imputing missing radiological data.
Discrimination: ability to discriminate between those with and without the outcome		
C-statistic	Assessing pairs of patients where one has more severe outcome than the other, it reflects the fraction of patients where those with the more severe outcome have higher predictions than those with the less severe outcome [24].	Linear regression analyses where the predicted $\Delta SHS_{0\text{-}6years}$ is compared with the observed $\Delta SHS_{0\text{-}6years}$ categorized in no/little, moderate and severe progression ($\Delta SHS_{0\text{-}6years}$ ≥6, 7-30 and >30).
Calibration: agreement between observed and predicted outcomes		
Calibration	Scatterplot with predicted outcome on the x-axis and observed outcome on the y-axis.	Scatter plot of observed versus predicted progression over six years ($\Delta SHS_{0\text{-}6years}$), both as continuous outcomes.
Reclassification: ability to reclassify patients by adding predictors to the model		
Net correct reclassification	Comparing the predicted classification with the observed classification when using two models; assessed is the net change in the correct direction (correct minus incorrect reclassifications).	Linear regression analyses in which the predicted $\Delta SHS_{0\text{-}6years}$ is calculated. Then both the observed and predicted $\Delta SHS_{0\text{-}6years}$ are categorized in no/little, moderate and severe progression ($\Delta SHS_{0\text{-}6years}$ ≥6, 7-30 and >30). Analyses were done on patients with complete data (n=239) and on all patients (n=426) when imputing missing radiological data.

R^2=proportion of explained variance; $\Delta SHS_{0\text{-}6years}$=progression in Sharp-van der Heijde score over six years

The ARH1 covariance matrix was used as suggested previously by Knevel et al [22]. Valuable of this repeated measurement analysis (RMA) is that it takes advantage of within-patient correlations of serial X-rays and allows the inclusion of patients with missing X-rays at certain time-points (figure 1). The improved fit of the model when adding treatment effects, traditional risk factors, genetic risk factors or combinations of these to a model consisting of only time effect was measured as the relative increase in the Nagelkerke R^2 between the models with the risk factors of interest and with only the time effect [23]. Importantly, this is not a direct measure of the explained variance, which cannot be determined in RMA such as linear mixed model analysis. Therefore, the R^2 was subsequently determined in linear regression analyses.

R^2 - This reflects the absolute proportion of the variance that is explained by the factors in the model and was determined using linear regression analyses with $\Delta SHS_{0-6\ years}$ as outcome. A limitation of this outcome is that only patients with complete follow-up could be studied (figure 1). Regression models were fitted that included treatment effects, traditional risk factors, genetic risk factors or combinations of these. Because adding more variables to a model will increase the fit of a model and thus the R^2, the adjusted R^2 was also calculated. This includes a correction for the number of variables in the regression model.

C-statistic - Harrel's C-statistic was assessed as described in the online supplementary methods [24]. It reflects the accuracy of discriminating patients with and without the outcome and does not reflect the absolute risk on an outcome. For clinical risk prediction it is more relevant that a new model can more accurately stratify individuals into risk categories. Hence, calibration (agreement between observed and predicted outcomes) and reclassification have gained popularity [25,26].

Calibration and Reclassification - First, the observed ΔSHS0–6 years was plotted against the $\Delta SHS_{0-6\ years}$ that was predicted by linear regression models including treatment effects and traditional factors or including treatment effects, traditional and genetic factors (calibration plot). Then the actual observed $\Delta SHS_{0-6\ years}$ and the predicted $\Delta SHS_{0-6\ years}$ were categorised in three severity groups ($\Delta SHS_{0-6\ years}$ ≤6, 7–30 and >30 units). To assess the improvement in predictive performance gained by adding genetic information to the prediction model, the proportion of patients that was correctly reclassified (correct reclassifications minus incorrect reclassifications) was determined. This was done for the total population and for each severity group separately.

Sensitivity analyses

The R^2 depends on the variance of the outcome. Therefore, the R^2 may change in case other follow-up durations are studied. To assess the influence of this effect, the R^2 was also determined for radiologic progression over 4, 5 and 7 years ($\Delta SHS_{0-4\ years}$, $\Delta SHS_{0-5\ years}$ and $\Delta SHS_{0-7\ years}$).

Only 239 of the 426 patients had complete radiologic data till year 6. As missing was not completely at random, we repeated the linear regression analyses with missing radiologic data imputed. We performed single conditional mean imputation by replacing missing values with the values predicted by the RMA with SHSs over 7 years of disease as outcome, and time and all traditional and genetic risk factors as variables. Subsequently, the R^2 and reclassifications were again determined.

Some of the genetic variants were identified as risk factors for radiologic progression in ACPA-positive RA, the more severe subset of RA. Therefore, the analyses of R^2 and reclassifications with $\Delta SHS_{0-6\ years}$ as outcome were repeated in the ACPA-positive subgroup (n=144).

Analyses were performed using SPSS V.20.0 and Stata V.12.

RESULTS

Patients and traditional risk factors

Patient characteristics are presented in table 3. The median SHS at baseline was 5.0 (IQR 2.0–10.0) and at year 6 it was 22.3(IQR 9.0–47.0); the median $SHS_{0-6\ years}$ was 14.0 (IQR 4.5–39.0). Treatment effects explained 7.1% of the variance of radiologic progression over 6 years (ΔSHS0–6 years). The R^2 of the individual traditional risk factors, determined in univariable regression analyses showed the highest values for ACPA and RF (R2 22.8% and 19.4%, respectively, table 3). All traditional risk factors together explained 31.2% of the variation and treatment effects and traditional risk factors combined explained 36.5% (figure 2A, see online supplementary table S2A). The adjusted R^2s were respectively 28.5% and 33.7% (figure 2D).

Genetic risk factors

Improved fit

First, all radiologic data of 426 patients were assessed using RMA. Models without and with genetic risk factors were compared, revealing that the model including the genetic risk factors had a 3.2% better fit in predicting radiologic progression com-pared to a model including only treatment effects and traditional risk factors (see online supplementary table S1). Since this measure is difficult to interpret, we continued with determining the R^2.

R^2

The R^2 of individual genetic risk factors was determined in univariable analyses (table 1). Rs11908352 in MMP-9 and the human leucocyte antigens-shared epitope (HLA-SE) alleles had the largest R^2 (4.7% and 4.0%, respectively). All genetic risk factors together explained 18.1% of the variance in $\Delta SHS_{0-6\ years}$ (figure 2B). The adjusted R^2 was 11.8% (figure 2E). Next, it was studied to what extent the genetic risk factors increased the R^2 compared to a model including treatment effects and traditional risk factors. A model including all factors (treatment effects, traditional and genetic risk factors) resulted in an R^2 of 43.9% and adjusted

Table 3. Characteristics of patients and the R^2 of each individual characteristic for radiologic progression over six years.

	All patients (n=426)	R^2 $\Delta SHS_{0\text{-}6years}$ (%) in RA (n=239)	R^2 $\Delta SHS_{0\text{-}6years}$ (%) ACPA-pos RA (n=144)
Age, mean (sd), years	56.6 (15.3)	<0.1	<0.1
Female gender, n (%)	290 (68.1%)	1.7	0.9
Symptom duration at first visit, median (IQR), months	4.4 (2.4-8.6)	2.3	0.7
Localization initial joint symptoms		<0.1	0.1
Upper extremities, n (%)	204 (47.9%)		
Lower extremities, n (%)	57 (13.4%)		
Upper and lower extremities, n (%)	165 (38.7%)		
66-SJC, median (IQR), n	8 (4-14)	2.0	<0.1
BMI, median (IQR), n	25.4 (23.0-27.6)	3.1	1.8
ACPA-positive, n (%)	227 (53.3%)	22.8	-
IgM-RF positive, n (%)	248 (58.2%)	19.4	0.3
ESR, median (IQR), mm/h	33.0 (18.0-55.0)	2.7	2.0

The presented R^2 s were based on univariable analyses of each individual risk factor. 239 patients of the total included 426 patients completed follow-up until six years, 144 of these patients were ACPA-positive. R^2=proportion of explained variance; $\Delta SHS_{0\text{-}6years}$=progression in Sharp-van der Heijde score over six years.

R^2 of 36.7%. As the R^2 of the model, including treatment and traditional risk factors was 36.5%, the increase in the R^2 by genetic risk factors was 7.4% (p-value R^2_{change}=0.056, figure 2C, see online supplementary table S2A). When comparing adjusted R^2s, genetic factors increased the R^2 with 3.0% (figure 2F).

C-statistic

The C-statistic increased from 0.78 (95% CI 0.73 to 0.82) for a model with treatment and traditional factors to 0.82 (95% CI 0.77 to 0.86) for a model including treatment, traditional and genetic factors.

Calibration and reclassification

Observed progression rates were plotted against predicted progression rates by a linear regression model with treatment effects and traditional risk factors as variables. When categorising patients in three groups ($\Delta SHS_{0\text{–}6\ years}$ ≤6, 7–30 and >30 units) 134 of 239 patients (56.1%) were correctly classified. When genetic factors were added, 148 out of 239 patients (61.9%) were correctly classified by the model (figure 3B,C). Hence in total this concerned a net increase in correctly classified patients (proportion of correct reclassifications) of 5.9% (95% CI 3.2 to 9.6%). Evaluating the reclassifications per severity group, showed no net change for the group with no/little progression, a 5.1% net increase in correctly classified

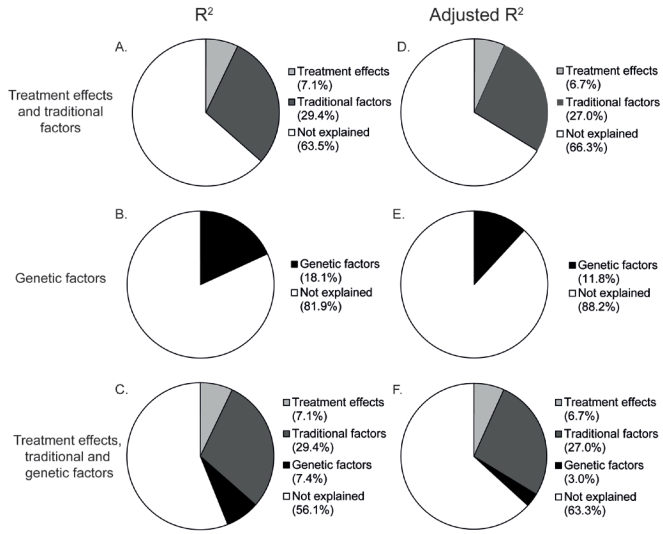


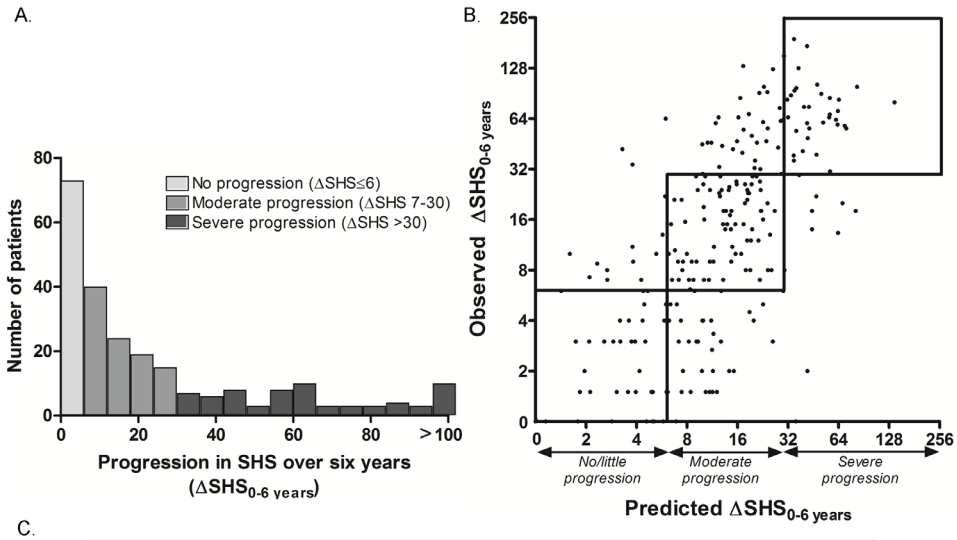
Figure 2. (A-C) Proportions of explained variance (R^2) (D–F) and adjusted explained variance (adjusted R^2) in progression in Sharp-van der Heijde score over six years ($\Delta SHS_{0-6\ years}$) by treatment effects and traditional risk factors (A and D), genetic risk factors (B and E), and treatment effects, traditional and genetic risk factors (C and F). The treatment strategy differed for different inclusion period. Therefore, the effects of treatment were determined before adding traditional and genetic factors (A, C, D and F). The studied traditional risk factors are presented in table 3 and included age, gender, symptom duration at first visit, localisation initial joint symptoms, 66-SJC, BMI, ACPA-positivity, RF-positivity and ESR. The studied genetic risk factors are presented in table 1 and included genetic variants in *HLA-DRB1, CD40, IL-15, DKK-1, IL2RA, GRZB, IL-4R, SPAG16, C5orf30, MMP-9* and *OPG*. The data presented are based on the patients with complete data over six years (n=239). Analyses on all patients after imputation of missing data (n=427) revealed similar results, see online supplementary table S2B. The p-value for change in R^2 after adding genetic factors was 0.056 for patients with complete data.

patients for the group with moderate progression, and a 13.2% net increase in correctly classified patients for the group with severe progression (figure 3C, see online supplementary table S3). Thus, the proportion of patients that was correctly reclassified when adding genetic factors increased in particular in the most severe patient group.

Sensitivity analyses

To check for consistency, $\Delta SHS_{0-4\ years}$, $\Delta SHS_{0-5\ years}$, and $\Delta SHS_{0-7\ years}$ were also assessed as outcomes. Adding genetic risk factors to a model with treatment effects and traditional risk factors yielded an increase in R^2 of 5.5% for $\Delta SHS_{0-4\ years}$ (p-value $_{R^2\ change}$=0.085), 7.1% for $\Delta SHS_{0-5\ years}$ (p-value $_{R^2\ change}$ =0.035) and 9.8% for $\Delta SHS_{0-7\ years}$ (p-value $_{R^2\ change}$=0.026) (see online supplementary table S4).

When missing SHSs were imputed and all 426 patients were studied, the increase in R^2 when adding genetic factors to a model with treatment and traditional risk factors and $\Delta SHS_{0-6\ years}$ as outcome was 5.3% (p-value $_{R^2\ change}$=0.001) (see online supplementary table S2B). The net proportion of patients that was correctly reclassified was 5.4% (95% CI 3.5 to 8.0%); for the groups with no/little, moderate and severe progression these were respectively 1.3%, 6.6% and 9.5%. The proportion of correctly classified patients was 286/426 (67.1%) (see



C.

	Observed progression over 6 years			
	No (ΔSHS ≤6)	Moderate (ΔSHS 7-30)	Severe (ΔSHS >30)	Total
Predicted progression over 6 years by model without genetic risk factors				
No (ΔSHS ≤6)	**34**	15	1	50
Moderate (ΔSHS 7-30)	34	**75**	42	150
Severe (ΔSHS >30)	4	9	**25**	39
Predicted progression over 6 years by model with genetic risk factors				
No (ΔSHS ≤6)	**34**	11	3	48
Moderate (ΔSHS 7-30)	36	**80**	31	147
Severe (ΔSHS >30)	2	8	**34**	44
Total	72	99	68	239

Figure 3. (A) Distribution of observed progression in Sharp-van der Heijde score over six years ($\Delta SHS_{0-6\ years}$), (B) observed versus predicted $\Delta SHS_{0-6\ years}$ by a model consisting of treatment effects, traditional and genetic risk factors and (C) numbers of patients per categorized observed and predicted $\Delta SHS_{0-6\ years}$ by models without and with genetic risk factors, resulting in the net proportion of correct reclassifications. (B) The dots in the boxes represent the 148 of the 239 patients in whom the severity of radiologic progression over six years was correctly predicted by the model, including treatment effects, traditional and genetic risk factors. (C) The model without genetic risk factors correctly classified 134 of 239 patients (56.1%) and the model with genetic risk factors correctly classified 148 of 239 patients (61.9%), resulting in a total net proportion of correct reclassifications of 5.8% (95% CI 3.2 to 9.6%). Evaluating reclassifications per severity group showed, respectively, no net change (5 correct and 5 incorrect reclassifications, 0/72), a 5.1% net increase (10 correct and 5 incorrect reclassifications, 5/99) and a 13.2% net increase (14 correct and 5 incorrect reclassification, 9/68) in correct classifications for the groups with no/little, moderate and severe progression (see also online Supplementary table S3).

online supplementary table S4).

In the subset of ACPA-positive patients, the median SHS at year 6 was 32.5 (IQR 17.3–65.8), and the median $\Delta SHS_{0-6\ years}$ 24.0 (IQR 10.6–57.5). The genetic factors together explained 17.1% of the variance in $\Delta SHS_{0-6\ years}$. Adding genetic factors to a model already including treatment effects and traditional risk factors increased the R^2 with 15.1% (p-value

R^2_{change}=0.11, see online supplementary table S5). The net proportion of correctly reclassified patients was 4.9% (95% CI 2.0 to 9.8%); for three severity groups, these were 0%, 3.1% and 8.6%. The model including all factors classified 91/144 (63.2%) of the ACPA-positive patients correctly (see online supplementary table S6).

DISCUSSION

New genetic risk factors for radiologic progression in RA have been identified recently. This study evaluated how much of the variance in radiologic progression is explained by these genetic factors together and whether these genetic factors improve predicting the severity of the disease course. We observed that genetic risk factors together explained 12–18% of the variance in joint destruction, and that adding the genetic factors to a prediction model already consisting of treatment effects and traditional risk factors resulted in a net increase of correctly classified patients of 6%. This increase was largely due to improved identification of patients with severe progression. Based on the Icelandic RA population, the heritability of radiologic progression was estimated at 45–58% [2]. Our observation that studied genetic factors explained around 18% suggest that part of the heritability is still missing. Several explanations may account for this. Part of the relevant genetic variants may still be unidentified or gene-gene interactions may play a role. The heritability in the Icelandic and Dutch RA population may also be dissimilar, prohibiting a direct comparison of percentages.

Adding genetic factors to a model with known risk factors had a small but independent contribution (3–7%) to the explained variance in radiologic progression. An explanation that this increase is less than the 12–18% of variation found for genetics alone is that part of the genetic factors are associated with traditional risk factors that were already included in the model. Probably these genetic factors relate to the outcome by mediating through these traditional risk factors and, therefore, they do not contribute to the model when the intermediate risk factors are also included. This observation differs from previous observations done for RA susceptibility where identified genetic susceptibility factors did not contribute independently to predicting the development of RA using a model with traditional factors, among which is ACPA [27]. The variants that had the largest independent contribution to the increase in R^2 were rs1528873 (*DKK-1*), rs7607479 (*SPAG16*) and rs11908352 (*MMP-9*) (data not shown). Intriguingly, all these proteins are involved in bone metabolism or cartilage destruction, processes that were not represented by the assessed traditional factors. Notably, due to the strong correlation between ACPA and HLA-SE, adding only HLA-SE to a model already containing ACPA was not helpful (R^2 change 0.1%, p-value R^2_{change}=0.63). Conversely, the R^2 change when adding the non-HLA variants to the model including traditional factors was 7.3% (p-value R^2_{change}=0.045).

The existing prediction matrices for rapid radiologic progression consist of a few traditional risk factors, were developed in a selected set of severe RA patients, could not adequately classify ~50% of the patients and had difficulties with identifying the patients

with severe progression in particular [8–13]. We evaluated nine traditional factors in a general population of RA patients, and observed that also here, 46% of RA patients were incorrectly classified. When evaluating traditional and genetic factors 62% of RA patients were correctly classified and 38% misclassified. Assuming that clinicians prefer to have at least 80% of the patients correctly predicted, the derived models including genetic variants were still insufficient for use in clinical practice. Importantly, with the help of genetic factors, the correct identification of especially those RA patients with severe radiologic progression increased.

We have chosen to study genetic variants that were replicated in independent studies or found significant in meta-analysis including all published data. Variants that were associated with radiologic progression in only one or two cohorts but not replicated or significant in meta-analyses were not included [28]. Potentially, future research will reveal more severity factors for RA and might increase the predictive accuracy.

Because of the negative implication of our conclusion, we did not seek for external validation or internal validation using cross-validation. The observed R^2 values may have been overestimated as many variables were included. Controlling for overfitting was done by determining the adjusted R^2 (correcting for the number of variables). However, some variables were correlated (for SNPs the correlation coefficients were <0.8) and, consequently, the correction may have been too stringent and the adjusted R^2 values underestimated. Presumably, the actual explained variance lies between the presented R^2 and adjusted R^2 values.

Several sensitivity analyses were done to check for the consistency of the results on the R2. Because missing radiologic data may be due to selection bias, analyses were also repeated after imputation of missing radiographs. Nonetheless, the consistent results in all sensitivity analyses indicate the reliability of our results.

Because some of the genetic risk factors were identified in ACPA-positive RA, we also performed subanalyses on ACPA-positive patients. Compared to the total RA population, the R^2 of the traditional risk factors was smaller (this may be explained by absence of the effect of ACPA) and the increase in R^2 when adding genetic factors was larger. Importantly, the R^2 values between the total and ACPA-positive population cannot be directly compared, as the total variance in $\Delta SHS_{0\text{–}6\ years}$ differed. The number of ACPA-positive patients was relatively small, providing another limitation.

In conclusion, all genetic severity factors together explained 12–18% of the variance in radiologic progression. Additional use of genetic factors resulted in increased correct classification of patients in severity risk groups. Nonetheless, 38% of the patients were still not correctly classified. Therefore, we considered the predictive performance of the derived prediction model insufficient for use in clinical practice.

SUPPLEMENTARY DATA

Supplementary data are published on the website of the *Annals of the Rheumatic Diseases*.

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Does a genetic variant in *FOXO3A* predict a milder course of rheumatoid arthritis?

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The severity of rheumatoid arthritis (RA) is variable between patients, and the processes underlying these interindividual differences are scarcely understood. Although it has been observed that the severity of joint destruction is heritable [1], and several identified genetic risk factors have been replicated in independent cohorts [2,3], a large part of the total genetic effect is still unexplained. Unraveling the biologic processes that determine the course of RA increases our comprehension of disease progression and may convey novel targets for focused therapies.

Lee et al reported a milder disease course in patients carrying the *FOXO3A* minor allele (G) of rs12212067 [4]. That candidate gene study addressed genetic variants in the immune pathways of interleukin-2 (IL-2) and IL-7 and was initially performed in patients with Crohn's disease. It was observed that the rs12212067 minor allele was associated with higher transcription of FoxO3 in blood monocytes after lipopolysaccharide stimulation and with down-regulation of tumor necrosis factor α (TNFα) and other proinflammatory cytokines and up-regulation of IL-10. The relevance of FoxO3 in disease outcome was supported by associations with more severe malaria and less severe joint damage in patients with early RA[4].

FoxO3 is a transcription factor that is involved in the regulation of immune cell homeostasis [5]. Increased expression of FoxO3 in polymorphonuclear cells has been described in RA [6]. The results described by Lee et al are promising, because identification of predictors of interindividual differences in disease outcome is the Holy Grail of personalised medicine. This finding therefore requires replication in independent cohorts. The challenges within the field of RA severity are that large longitudinal cohorts with well-characterised data are scarce, and that cohorts of patients who were treated in an era when disease-modifying antirheumatic drugs (DMARDs) were less potent and strategies were not guided by the Disease Activity Score (DAS) are rare [7]. We examined rs12212067 in *FOXO3A* in relation to the severity of RA in multiple cohorts; the majority of patients studied were treated in the era before the introduction of biologic agents and DAS-guided therapy.

The main outcome of our study was radiographic progression. Five independent data sets were studied, comprising a total of 2,300 patients with RA and 5,512 radiographs. RA was defined according to the American College of Rheumatology 1987 revised criteria [8]. All patients gave informed consent, and approval was obtained from the local medical ethics committees.

The Leiden Early Arthritis Clinic (EAC) cohort comprised 597 patients with early RA, all of whom were included between 1993 and 2006 [9]. At baseline and at yearly follow-up visits over 7 years, a total of 3,143 sets of radiographs of the hands and feet were obtained. These radiographs were scored according to the Sharp-van der Heijde (SHS) method [10] by one reader in chronologic order (within-reader intraclass correlation coefficient [ICC] 0.91). The initial treatment strategy differed for different inclusion periods: patients included in 1993–1995 were initially treated with nonsteroidal anti-inflammatory drugs, patients included

in 1996–1998 were initially treated with hydroxychloroquine or sulfasalazine, and patients included in 1999–2006 were promptly treated with methotrexate [9].

The Umeå cohort consisted of 459 patients with early RA from Sweden, in whom RA was diagnosed between 1996 and 2010 [11]. A total of 868 sets of radiographs of the hands and feet obtained at baseline and year 2 were scored using the Larsen method [12], as described previously [11]. All patients were initially treated with methotrexate or sulfasalazine. Treatment with biologic agents during the 2-year follow-up period was uncommon (5.7%).

The North American Rheumatoid Arthritis Consortium (NARAC) study group comprised 384 unrelated patients in whom RA was diagnosed between 1953 and 2002 [13]. One set of radiographs of the hands was available for each patient. The radiographs were scored according to the SHS method (ICC 0.99).

The Wichita cohort consisted of 101 patients from a single practice in Wichita, Kansas, in whom RA was diagnosed between 1963 and 1999 [14]. Radiographic evaluations were not performed at protocolised time points; 358 sets of hand radiographs were obtained during the first 15 years after disease onset. These were scored in a known time order, using the SHS method (ICC 0.98).

The National Data Bank for Rheumatic Diseases (NDB) cohort comprised 759 patients from the US and Canada, in whom RA was diagnosed between 1944 and 1999 [15]. One set of radiographs of the hands was available for each patient. The radiographs were scored using the SHS method (ICC 0.98). The patients in the 3 North American cohorts were treated in an era when biologic agents were uncommon.

Genotyping in the Leiden EAC, Umeå, Wichita, and NDB cohorts was performed using the Illumina ImmunoChip according to the manufacturer's protocols, as previously described [16], and data for rs12212067 located on chromosome 6 were extracted. In the NARAC cohort, genotyping was performed using Illumina HapMap 500 BeadChips [13,17]. Data for rs12212067 were not available in the NARAC group, but data for a perfect proxy for this variant (rs11153120) were retrieved (r^2 1.00).

In all data sets, the radiographic scores were log-transformed before analyses to approximate a normal distribution. In each data set, the relative progression rate in patients with the rs12212067 minor allele was estimated, using patients with the common genotype as reference. An additive model was used. For the analyses in the cohorts with multiple measurements per patient (Leiden EAC, Umeå, and Wichita), a multivariate normal regression analysis was performed, with radiographic damage as the response variable [18]. For the data sets with one radiographic measurement per patient (NARAC and NDB), the estimated yearly progression rate (the total SHS divided by the number of disease-years at the time of radiography) was studied. Details on the statistical methodology and adjustment factors are available in the online Supplementary Methods (available on the Arthritis & Rheumatology website). Because all of the obtained effect sizes represented the relative

increase in the progression in joint destruction per year per minor allele, the effect sizes and standard errors of the individual analyses could be compared and combined in an inverse-variance weighted meta-analysis.

Because of the observation that the rs12212067 minor allele was associated with lower TNFα expression and higher production of anti-inflammatory interleukins [4], which presumably affect not only joint damage but also arthritis persistency, a second outcome measure, achieving sustained DMARD-free remission, was studied. Sustained DMARD-free remission is defined as the sustained absence of clinically detectable arthritis after discontinuation of DMARD therapy. This is described elsewhere and in the online Supplementary Methods. This stringent definition of remission is a proxy for cure [19]. Data on achieving DMARD-free remission were available only in the Leiden EAC cohort during 15 years of follow-up. Kaplan-Meier survival analyses were performed.

The characteristics of the patients are shown in the online Supplementary Table 1. In the largest cohort, consisting of 3,143 radiographs and 597 patients, rs12212067 was not statistically significantly associated with the severity of radiographic progression (p=0.14). No tendency toward less severe joint damage in the presence of the minor G allele was observed (Figure 1A). Significance was not obtained in any of the other cohorts. The meta-analysis did not reveal a significant association with radiographic progression (p=0.10), and the directionality of the effect sizes was not uniform (Figure 1B). When the secondary outcome of achieving sustained DMARD-free disease remission was evaluated, no significant association was observed (p=0.54) (Figure 1C).

Although the analyses described by Lee et al were performed in a mixed autoantibody-positive and antibody-negative population [4,20,21], we also performed analyses adjusted and stratified for the presence of anti–citrullinated protein antibodies (ACPA). In the analyses that were additionally adjusted for ACPA, rs12212067 was not significantly associated with the severity of radiographic progression (see online Supplementary Figure 1A). In the meta-analysis of the ACPA-positive subgroup, a significant result was obtained using the fixed-effects model but not the random-effects model; the directionality of the effect sizes was diverse (see online Supplementary Figure 1B). In the meta-analysis of the ACPA-negative subgroup, no association between rs12212067 and radiographic progression was observed. For the outcome of achieving sustained DMARD-free disease remission, no significance was observed in the ACPA-positive and ACPA-negative strata (p=0.77 and p=0.62, respectively).

In conclusion, from a clinical perspective, it is highly relevant to unravel the biology determining disease outcome. A recent study demonstrated a protective association between a genetic variant in *FOXO3A* and the severity of radiographic joint destruction in 2 early RA cohorts consisting of both ACPA-positive and ACPA-negative patients [4,20,21]. Using 5 independent RA cohorts, we could not replicate an association of *FOXO3A* with the severity of RA, implying that the initial observation in UK cohorts cannot be extrapolated to other

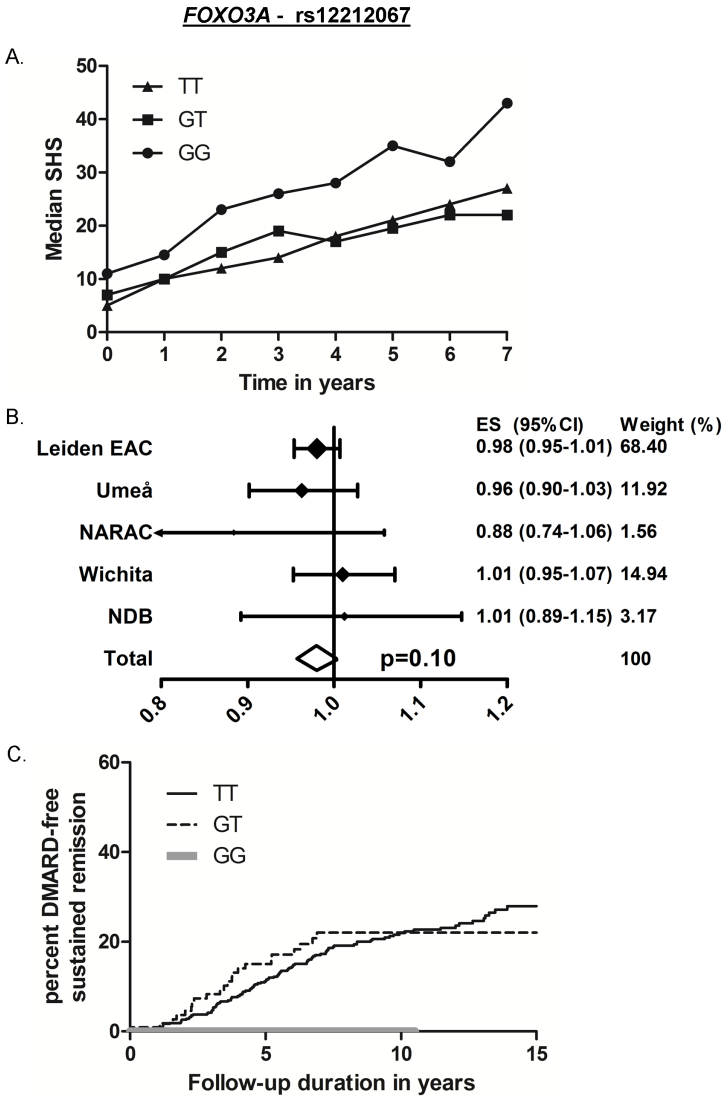

Figure 1. Relationship of *FOXO3A* rs12212067 genotypes with radiographic progression and DMARD-free sustained remission. (A) Median raw Sharp-van der Heijde (SHS) scores during 7 years of follow-up in patients in the Leiden EAC cohort. (B) Inverse-variance weighted meta-analysis of the annual radiographic progression rate in 5 cohorts, consisting of 2,300 patients and 5,512 radiographs (I^2 0.0%, p=0.59; for both the fixed-effects and random-effects models, p=0.10). (C) Frequency of achieving sustained DMARD-free remission in the Leiden EAC cohort (p=0.54 by log rank test). Only 6 patients had the GG genotype, and none of them achieved disease remission. The frequencies of the minor allele (G) were 9.3% in the Leiden EAC cohort, 12.0% in the Umeå cohort, 8.6% in the NARAC cohort, 9.3% in the Wichita cohort, and 10.5% in the NDB cohort. ES=effect size; 95% CI=95% confidence interval.

populations. This may reflect some differences in regulating progression in very early disease, which was the focus of the UK studies. Some data sets studied here were smaller than the UK cohorts; consequently, these individual cohorts were underpowered to replicate the signal individually. However, when the 5 data sets were combined, the number of radiographs was larger than that in the original study, and the meta-analysis was adequately powered to identify statistically significant differences and prevent false-negative results. The prevalence of ACPA in our cohorts was similar or higher than that in the original cohorts [20,21]. After stratification for ACPA, the p-value for a meta-analysis with a fixed-effects model was less than 0.05 within ACPA-positive patients; however, the directionality of the effect sizes was variable. The question of whether SNP rs12212067 in *FOXO3A* is associated with joint destruction in the ACPA-positive subgroup of patients with RA requires further investigation. The absence of an association of rs12212067 with sustained DMARD-free remission (the reverse of disease persistency) further supported the notion that *FOXO3A* is not a major factor regulating the severity of the course of RA.

SUPPLEMENTARY DATA

Supplementary data are published on the website of *Arthritis & Rheumatology*.

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SPP1 rs9138 variant contributes to the severity of radiological damage in anti-citrullinated peptide autoantibody-negative rheumatoid arthritis

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ABSTRACT

Objective

We recently reported an association of the *SPP1* rs9138 and rs11439060 functional variants with the risk of rheumatoid arthritis (RA), the association being greater in anti-citrullinated peptide autoantibody (ACPA)-negative patients. We hypothesised that *SPP1* may contribute to the severity of joint destruction in RA, specifically in the ACPA-negative population.

Methods

Patients with RA in the ESPOIR cohort underwent genotyping for *SPP1* rs9138 and rs11439060. Radiographs of the hands and feet were obtained at the first visit and at 1- and 2 year follow-up. Association analyses were performed by ACPA-status. A replication study of the relevant subset of the Leiden Early Arthritis Clinic (EAC) cohort was performed.

Results

In the ESPOIR cohort (652 patients), rs9138 was significantly associated with radiological progression of joint destruction at 2 years, the association being restricted to 358 ACPA-negative patients (p=0.034). In the replication study within the Leiden EAC cohort (273 ACPA-negative patients), rs4754, which is in complete linkage disequilibrium with rs9138, was significantly associated with joint damage progression in ACPA-negative patients at 2 and 7 year follow-up (p=0.019 and p=0.005, respectively). Combined analysis of the two cohorts revealed a 0.95 fold rate of joint destruction per year per minor allele (p=0.022).

Conclusions

The *SPP1* rs9138 variant contributes to joint damage progression in ACPA-negative RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic, inflammatory, autoimmune disease characterised by peripheral synovial joint inflammation which can lead to joint destruction. Approximately two-thirds of RA cases are seropositive for rheumatoid factor or anti-citrullinated peptide autoantibodies (ACPA) [1]. The heritability of ACPA-positive and ACPA-negative disease is comparable [2], and recent association studies have provided further support for distinct genetic aetiologies of ACPA-positive and ACPA-negative RA subsets [3,4]. ACPA-positive RA patients are particularly characterised by progressive joint destruction [5]. However, little information is available on joint destruction processes in the ACPA-negative subset. Currently, we cannot offer personalised medicine for patients with RA because we cannot identify those who will have the most severe disease course, nor do we understand the pathogenesis underlying these interindividual variances. To improve this situation, identification of risk factors for joint destruction is required.

Genetic variants are estimated to contribute to 58% of the total variance in RA joint destruction, with clinical and serological risk factors explaining only about one-third of the total phenotypic variation [6]. Most risk alleles for RA joint destruction have been identified in ACPA-positive patients or in pooled ACPA-negative and ACPA-positive patients, but we lack information about the genetic contribution to ACPA-negative RA severity [7–9]. Identifying individual genetic risk factors would increase our understanding of the mechanisms underlying variation in severity of joint destruction, particularly in ACPA-negative disease.

Recently, through a large case-control association study, our group reported a significant contribution of the combination of the *SPP1* rs11439060 and rs9138 frequent alleles to risk of RA, the magnitude of the association being greater in ACPA-negative patients [3]. These patients fulfilled the 1987 American College of Rheumatology (ACR) revised criteria for RA, which include radiographic changes typical of RA [10]; so ACPA-negative individuals may have had joint destruction to be classified as having RA. *SPP1* encodes osteopontin (OPN), an extracellular-matrix glycosylated phosphoprotein with multiple functions including bone formation and remodelling [11]. Consequently, we hypothesised that *SPP1* variants may contribute to the severity of joint destruction in RA, specifically in ACPA-negative patients.

PATIENTS AND METHODS

Study population

The exploratory study included 652 patients with RA from the ESPOIR cohort who were positive or negative for ACPA and were included in the large case-control association study previously reported [3,12]. The replication study included 273 ACPA-negative RA cases from the Leiden Early Arthritis Clinic (EAC) cohort (table 1) [13]. All patients fulfilled the 1987 ACR revised criteria for RA [10]. They all provided informed written consent as approved by the

Table 1. Characteristics of the ESPOIR and Leiden EAC cohorts of patients with RA genotyped for *SPP1* rs9138 or rs4574

Cohort	No of patients	No of sets of radiographs of hands and feet	Year of diagnosis	Follow-up (years)	Method of scoring	ICC	ACPA-negative (%)	Age (years), mean±SD	Female patients, n (%)
ESPOIR	652	1,768	2002–2005	2	SHS	0.97	54.9	47.9±12.2	500 (76.7)
Leiden EAC:	273	1,316	1993–2006	7	SHS	0.91	100	58.9±16.3	187 (68.5)
ACPA-negative									
population									

recruiting site review board at each of the affiliated institutions.

Genotyping

In the exploratory study with the ESPOIR cohort, *SPP1* rs11439060 and rs9138 variants were genotyped by use of a competitive allele-specific PCR system (Kaspar genotyping; Kbioscience, Hoddeston, UK) [3]. In the Leiden EAC cohort, *SPP1* rs4754, which is in complete linkage disequilibrium with rs9138 [3], was genotyped using Illumina Human CytoSNP-12V2.

Radiographic joint destruction

In both the ESPOIR and Leiden EAC cohorts, all radiographs of hands and feet were scored by the Sharp-van der Heijde score (SHS) by one experienced reader per cohort who was blinded to clinical, biological and genetic data [14]. The intraclass correlation coefficient was 0.97 and 0.91, respectively.

Statistical analysis

A multivariate regression analysis (MRA) was used, with radiographic damage as the response variable (see online supplementary text for a detailed description of the MRA). The analyses were performed with the genetic variable and its interaction with time in the model, reflecting a constant and a time-dependent effect of progression of joint damage, respectively [15].

To validate our a priori hypothesis (ie, the contribution of *SPP1* rs11439060, rs9138 and the rs11439060-rs9138 risk allele combination in the ACPA-negative population), we selected the best-fit model at the exploratory stage, as previously described, which was then replicated in the Leiden EAC cohort [3]. MRA of both ACPA-negative populations was used to assess the magnitude of the *SPP1* effect on radiographic joint destruction in early RA. Analyses used SPSS V.20.0.

RESULTS

Exploratory study

We analysed data for 652 patients with RA and 1768 radiographs. For a complete overview of the MRA of *SPP1* rs11439060, rs9138 and risk allele combination, see online supplementary table S1. Briefly, the best-fit model involved rs9138 (see online supplementary table S2). In agreement with our a priori hypothesis, rs9138 was significantly associated with radiographic progression over a 2 year follow-up in ACPA-negative patients, as seen by an additive model with a 0.93 fold rate of joint destruction per year per minor allele as compared with the wild-type (p=0.034) (figure 1A-C).

Replication study

We analysed data for 273 ACPA-negative patients in the Leiden EAC cohort and 704 radiographs over a 2 year follow-up. We replicate the contribution of rs9138, as MRA revealed that rs4754 was associated with radiographic progression over the 2 year follow-up with a 0.81 fold rate of joint destruction for each minor allele at any time compared with the wild-type (p=0.019). Analysis of 1,316 radiographs over 7 years of follow-up revealed a persistent effect of rs4754, with a 0.78 fold rate of joint destruction for each minor allele at any time compared with the wild-type (p=0.005; figure 2A).

Combined analysis of early RA during the 2 year follow-up

Data for 631 ACPA-negative patients and 1,664 radiographs were available for the combined analysis of the ESPOIR and Leiden EAC cohorts. MRA over the 2 year follow-up revealed a 0.95 fold rate of joint destruction per year per minor allele compared with the wild-type (p=0.022; figure 2B).

DISCUSSION

The rate of progression of joint damage in RA is highly variable and is associated with the

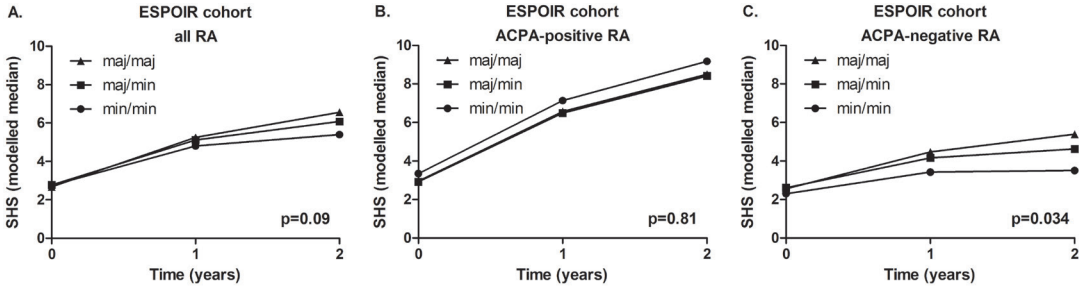

Figure 1. Association of *SPP1* rs9138 and joint damage progression in RA patients in the exploratory study. Multivariate regression analysis modelled median Sharp-van der Heijde scores (SHS) in patients with RA in the ESPOIR cohort (exploratory study) at 1 and 2 year follow-up. (A) Overall RA patients. (B) ACPA-positive RA patients. (C) ACPA-negative RA patients. Maj=major allele; min=minor allele.

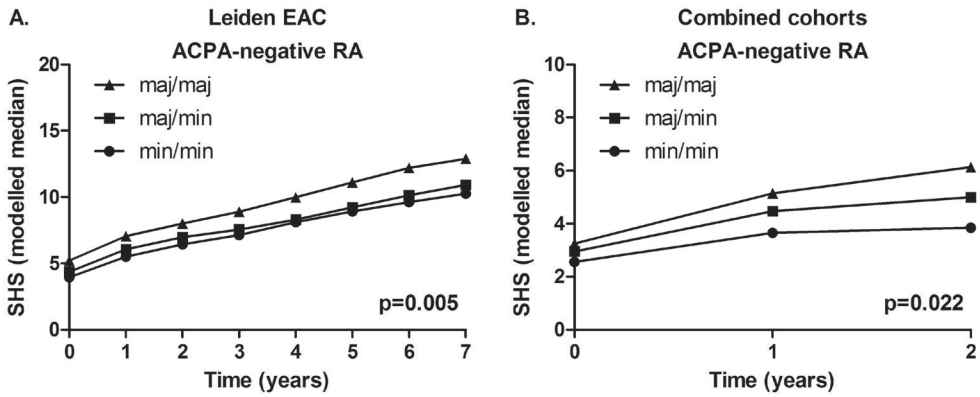

Figure 2. Association of *SPP1* rs4754 and joint damage progression in RA patients in the replication study and combined analysis at 2 year follow-up. (A) Multivariate regression analysis modelled median Sharp-van der Heijde scores (SHS) in patients with RA in the Leiden EAC ACPA-negative cohort during a 7 year follow-up (B) and combined analysis of the ESPOIR and Leiden EAC ACPA-negative cohorts at a 2 year follow-up. Maj=major allele; min=minor allele.

severity of the disease. Genetic variants are estimated to contribute to most of the local variance in RA joint destruction. Several data have suggested that OPN, encoded by *SPP1*, may be involved in bone erosion. In addition, we recently identified *SPP1* as a new RA susceptibility gene, the magnitude of the association being greater in ACPA-negative disease [3]. Because ACPA-negative patients fulfilled the ACR modified criteria, *SPP1* may contribute to the variation in joint destruction in this particular subset of the disease.

For the two *SPP1* rs11439060 and rs9138/rs4754 RA risk variants investigated, the rs9138/rs4754 common allele contributed to joint destruction of RA - that is, the minor allele had a protective effect. This finding is in agreement with our a priori hypothesis because the rs9138 common A allele has been identified as an ACPA-negative RA risk allele [3]. The replication study, including analyses at the same time of follow-up (2 years) and also after a longer period (7 years), provided evidence that the *SPP1* rs9138 variant contributes to the severity of radiographic damage in ACPA-negative RA in both the early and intermediate course of the disease. Analyses of the combined sets revealed an interaction between *SPP1* rs9138/rs4754 and time at the 2 year follow-up, which suggests a strong effect of *SPP1* on radiographic damage at the early stage of the disease. A recent study of the ESPOIR cohort found that the first-year radiographic progression was a predictor of further progression in early RA, which suggests that, after the early period of the disease, time has a constant effect [16].

Complex diseases, such as RA, invariably involve multiple genes and often exhibit variable symptom profiles. The extent to which disease symptoms, course and severity differ between affected patients may result from underlying genetic heterogeneity. Genes with modifier effects may or may not also influence disease susceptibility. Indeed, *SPP1* seems to act as a susceptibility and a modifier gene. The effect of the rs11439060 variant differed

in this study compared with our previous case-control study [3], the rs11439060-rs9138 risk allele combination not being identified as the best-fit model. Nonetheless, a contribution of rs11439060 could not be definitely excluded; the sample size required to detect such association with a power of 80% would be 1,000 ACPA-negative patients with early RA.

To our knowledge, this is the first report of the identification of a genetic variant associated with joint damage progression in ACPA-negative RA. We took advantage of two cohorts including sequential radiographs of hands and feet, which strengthened the evidence of the contribution of *SPP1* rs9138. Several studies have reported an association of the rs9138 A risk allele with low serum levels of soluble OPN [3,17]. However, to date, the exact role of OPN in RA joint damage is controversial: distinct murine models of RA have shown conflicting results on the relevance of OPN in bone erosion pathogenesis [18,19], and, more importantly, OPN blockade was found to be unlikely to induce robust clinical improvement in patients with RA [20].

In conclusion, we have identified and replicated a genetic *SPP1* variant predisposing to joint damage progression in ACPA-negative RA. Further studies of OPN at the protein level are required to better understand the role of this variant in the pathogenesis of the progression of radiographic damage in ACPA-negative RA.

SUPPLEMENTARY DATA

Supplementary data are published on the website of the *Annals of the Rheumatic Diseases*.

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A genetic study on *C5-TRAF1* and progression of joint damage in rheumatoid arthritis

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ABSTRACT

Introduction

The severity of joint damage progression in rheumatoid arthritis (RA) is heritable. Several genetic variants have been identified, but together explain only part of the total genetic effect. Variants in *Interleukin-6* (*IL-6*), *Interleukin-10* (*IL-10*), *C5-TRAF1*, and *Fc-receptor-like-3* (*FCRL3*) have been described to associate with radiographic progression, but results of different studies were incongruent. We aimed to clarify associations of these variants with radiographic progression by evaluating six independent cohorts.

Methods

In total 5,895 sets of radiographs of 2,493 RA patients included in six different independent datasets from the Netherlands, Sweden, Spain and North America were studied in relation to rs1800795 (*IL-6*), rs1800896 (*IL-10*), rs2900180 (*C5-TRAF1*) and rs7528684 (*FCRL3*). Associations were tested in the total RA populations and in anti-citrullinated peptide antibodies (ACPA)-positive and ACPA-negative subgroups per cohort, followed by meta-analyses. Furthermore, the associated region *C5-TRAF1* was fine-mapped in the ACPA-negative Dutch RA patients.

Results

No associations were found for rs1800795 (*IL-6*), rs1800896 (*IL-10*) and rs7528684 (*FCRL3*) in the total RA population and after stratification for ACPA. Rs2900180 in *C5-TRAF1* was associated with radiographic progression in the ACPA-negative population (p-value meta-analysis = 5.85×10^{-7}); the minor allele was associated with more radiographic progression. Fine-mapping revealed a region of 66Kb that was associated; the lowest p-value was for rs7021880 in *TRAF1*. The p-value for rs7021880 in meta-analysis was 6.35×10^{-8}. Previous studies indicate that the region of rs7021880 was associated with RNA expression of *TRAF1* and *C5*.

Conclusion

Variants in *IL-6*, *IL-10* and *FCRL3* were not associated with radiographic progression. Rs2900180 in *C5-TRAF1* and linked variants in a 66Kb region were associated with radiographic progression in ACPA-negative RA.

INTRODUCTION

Thanks to the introduction of novel treatments and up-to-date treatment strategies, the severity of joint destruction in rheumatoid arthritis (RA) has decreased considerably [1]. Nonetheless, in daily clinical practice radiographic progression is still prevalent and understanding the mechanisms underlying the inter-individual differences in radiographic progression is relevant. The heritability of joint destruction has been estimated to be 45% to 58% [2]. Thus far, several genetic risk factors for radiographic progression have been replicated in independent studies or found significant in meta-analyses of different cohorts, but together explain only 18% of variance in radiographic progression [3].

Part of the 'missing heritability' might be explained by not yet identified common genetic variants that associate with radiographic progression in RA. The literature on genetic variants for radiographic progression was reviewed recently [4]. Published and yet unpublished data were combined, and it was concluded that for 12 genetic variants their associations with radiographic progression were either replicated in independent cohort studies or found significant in meta-analysis of multiple cohorts. However, the associations between rs1800795 in *Interleukin (IL)-6*, rs1800896 in *IL-10*, rs2900180 in *C5-TRAF1* and rs7528684 in *Fc-receptor-like-3 (FCRL3)* and joint damage were not clear [4]. Rs1800795 in *IL-6* was associated with radiographic joint damage at baseline in 964 United Kingdom (UK) RA patients, but the association was mainly observed in autoantibody-positive patients [5]. *IL-10* was observed as a severity factor evaluating 138 RA patients [6], but not in a study of 108 RA patients [7]. Rs2900180 in *C5-TRAF1* was identified in a cross-sectional study [8]; it was also found significant in another UK cohort [9], but not in other datasets [4]. Rs7528684 in *FCRL3* was observed as a severity factor in two studies [10,11], although the association was once restricted to the subgroup with a disease duration of at least 10 years [11] and not found in other datasets [4,12].

Presumably, the scarcity of large well-defined longitudinal cohorts of RA patients who were treated in eras when early, tailored treatment and use of biologics were uncommon may have contributed to the incongruent findings.

In order to increase the comprehension on the associations of these variants with radiographic progression in RA and in the anti-citrullinated peptide antibodies (ACPA)-positive and ACPA-negative subgroups, we performed the present study and evaluated these genetic variants in six independent European and North American RA cohorts in one of the largest studies to date on RA severity.

METHODS

Study population

The six cohorts consisted in total of 5,895 sets of radiographs of 2,493 RA patients who

Table 1. Patient characteristics

	Leiden EAC	Umeå	HCSC-RAC	Wichita	NDB	NARAC	Total
Total number of patients	597	459	383	101	568	385	2,493
Total number of sets of radiographs	3,143	868	573	358	568	385	5,895
Radiographic follow-up in years*	7	2	10	15	NA	NA	
Disease duration in years at radiograph, mean (SD)**	NA	NA	NA	NA	10.1 (5.1)	13.9 (10.5)	
Method of scoring	SHS	Larsen	SHS	SHS	SHS	SHS	
Year of diagnosis	1993-2006	1995-2010	1976-2011	1963-1999	1980-1999	1953-2002	
Female, number (%)	402 (67.3)	321 (69.9)	293 (76.5)	70 (69.3)	444 (78.2)	281 (73.0)	
Age at diagnosis in years, mean (SD)	57.1 (15.6)	53.9 (14.5)	47.0 (14.0)	49.0 (11.7)	48.6 (12.7)	40.8 (11.9)	
ACPA-positive, number (%)[+]	309 (52.8)	339 (73.9)	165 (49.3)	97 (96.0)	453 (79.8)	385 (100)	
MAF rs1800795 (G) (*IL-6*), %	42.0	46.5	33.8	46.5	40.8	42.1§	
MAF rs1800896 (T) (*IL-10*), %	48.1	44.3	52.7	49.0	48.5	45.6	
MAF rs2900180 (A) (*C5-TRAF1*), %	36.0	36.4	27.8	36.6	35.6	39.0	
MAF rs3761959 (A) (*FCRL3*), %§§	45.5	44.2	42.2	50.0	49.7	47.4	

*For the studies with longitudinal radiographic data (more than one radiograph in time), the maximum radiographic follow-up duration was reported. ** For the studies with one radiograph per patient, the mean disease duration at time of the radiograph was reported. [+]ACPA status was missing in 12 patients from the Leiden EAC cohort, in 48 patients from the HCSC-RAC cohort and in 1 patient from the Wichita cohort. §Data on rs1800795 were not available in the NARAC; data on a proxy rs1554606 (R^2=0.868) were available. §§In all cohorts data on rs7528684 were not available; data on a perfect proxy rs3761959 (R^2=1.000) were available. MAF=minor allele frequency; NA=not applicable.

fulfilled the 1987 American College of Rheumatology (ACR) criteria (Table 1). All patients gave their informed consent and approval was obtained from the local Ethical Committee of each hospital.

Leiden Early Arthritis Clinic (EAC) - This cohort contained 597 Dutch early RA patients included between 1993 and 2006 [13]. At baseline and during yearly follow-up visits over seven years, 3,143 sets of hand and feet radiographs were made and chronologically scored by one experienced reader according to the Sharp-van der Heijde method (SHS) (within reader intraclass correlation coefficients (ICC) 0.91). The initial treatment strategy differed for different inclusion periods: patients included in 1993 to 1995 were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs), patients included in 1996 to

1998 were initially treated with hydroxychloroquine or sulfasalazine and patients included in 1999 to 2006 were promptly treated with methotrexate [13].

Umeå - This cohort involved 459 Swedish early RA patients included between 1995 and 2010. At baseline and after two years in total, 868 radiographs of hands and feet were made and scored using the Larsen score by two trained readers as described previously [14]. Treatment strategies differed between 1995 and 2000, 2000 and 2005 and 2006 and 2010, resulting in less severe radiographic progression in the subsequent treatment periods.

Hospital Clinico San Carlos - rheumatoid arthritis cohort (HCSC-RAC) - This Spanish cohort comprised 383 early RA patients, diagnosed between 1976 and 2011 [15]. During the first 10 years after disease-onset 573 radiographs of hands were made and scored chronologically according to the SHS (ICC 0.99). Initial treatment strategies differed for different inclusion periods: <1990 (initial treatment with NSAIDs), 1990 to 1999 (initial monotherapy conventional disease-modifying antirheumatic drugs (DMARDs), 2000 to 2004 (initial mono-therapy regularly and combination therapy rarely), 2005 to 2009 (initial combination therapy regularly used as well as biologics) and 2010 to 2011 (tailored treatment).

Wichita - This cohort comprised 101 patients from one practice in Wichita (KS, US) diagnosed between 1963 and 1999 [16]. In total, 358 sets of hand radiographs were made during the first 15 years after disease onset and scored with known time-order using the SHS (ICC 0.98).

National data bank for rheumatic diseases (NDB) - This dataset included 568 patients from the US and Canada, who were diagnosed between 1980 and 1999 [17]. One radiograph set of the hands was available per patient and SHS-scored (ICC 0.98).

North American Rheumatoid Arthritis Consortium (NARAC) - This dataset comprised 385 unrelated RA patients, who were diagnosed between 1953 and 2002 [18]. One radiograph set of the hands was available per patient. The radiographs were SHS-scored (ICC 0.99). The patients in the three North American cohorts developed RA in eras when early, tailored treatment and use of biologics were uncommon; no treatment effects were observed for different era of diagnoses.

Genotyping

In the EAC, Umeå, HCSC-RAC, Wichita and NDB cohorts genotyping was done using the Immunochip according to Illumina's protocols as described previously [19,20]. In the NARAC genotyping was performed using the Illumina Hapmap 500 BeadChip as described elsewhere [18]. Genotyping data were extracted of rs1800795 in *IL-6*, rs1800896 in *IL-10*, rs2900180 in *C5-TRAF1* and rs7528684 in *FCRL3*. Data on rs1800795 were not avail-able in the NARAC but genotyping data of a proxy rs1554606 (R^2=0.868 and D'=0.932) were retrieved. In all cohorts, data on rs7528684 (*FCRL3*) were not available; data on a perfect proxy rs3761959 (R^2 and D' both 1.000) were studied.

Fine-mapping

The *C5-TRAF1* region was fine-mapped in ACPA-negative patients of the EAC. Data of genetic variants in the region of rs2900180 were retrieved using the Immunochip, starting at the upstream haplotype block of *PHF19* until the downstream haplotype block of *C5* (chromosome 9: 122,680 Kb to 122,927 Kb). Genotypic data were accepted after quality control as described elsewhere [20], requiring minor allele frequency (MAF) >0.0001, Hardy-Weinberg equilibrium (HWE) p>0.001 and genotyping success rate >0.99. Genetic outliers and relatives (both defined by principal component analysis) and patients with a gender mismatch between the data file and DNA were excluded. In this way, 424 SNPs were obtained and analysed for their association with radiographic progression. The variant with the strongest association was subsequently associated with radiographic progression in the ACPA-negative patients of the Umeå, HCSC-RAC and NDB cohorts.

Downstream effect

To identify functional downstream effects of *C5-TRAF1*, a search was performed in publically available databases and datasets [21–26]. Explored were the RegulomeDB [21], datasets that have evaluated constitutive RNA expression by mapping expression quantitative trait locus (eQTL) in peripheral blood samples from 8,086 individuals [22] and purified CD4+ T-cells and monocytes from 461 individuals [23], and datasets that have evaluated response eQTLs (QTLs associated with change in expression after stimulation) on lymphoblastoid cell lines from 40 individuals [24], monocytes from 432 individuals [25] and monocytes derived dendritic cells from 534 individuals [26].

Statistical analysis

Associations between genotypes and radiographic joint damage were analysed per cohort using an additive model. In all datasets, radiographic scores were log-transformed (log10(radiographic score +1)) to approximate a normal distribution. The residuals of the used models were normally distributed around the zero-line in all cohorts, indicating a good fit of the models (Additional file 1).

In the cohorts with multiple sets of radiographs over time (EAC, Umeå, HCSC-RAC and Wichita) a multivariate normal regression model for longitudinal data was used with radiographic scores as response variable. This method takes advantage of the within-person correlation between repeated measurements; as such, the radiographic progression rates were estimated more precisely in the cohorts with serial radiographs compared to datasets with one radiograph per patient (for a detailed description see reference [27]. The obtained effect size (beta) was back-transformed to the normal score and indicated the fold rate of joint destruction per year per minor allele compared to the reference genotype.

In the cohorts with a set of radiographs at one time-point (NDB and NARAC) the estimated yearly progression rate was calculated (total SHS divided by number of disease

year at the time of the radiograph) in order to make the estimates of the progression rates comparable to those in the other datasets. A linear regression analysis was used with estimated yearly progression as outcome variable. Here, also, the obtained effect size was back-transformed and indicated the fold rate of joint destruction per year per minor allele compared to the reference common genotype.

In all datasets, adjustments were made for age and gender. In the cohorts that included patients in periods with different treatment strategies (EAC, Umeå and HCSC-RAC) analyses were also adjusted for the inclusion period as proxies for differences in treatment strategies.

The majority of datasets studied were estimated to be insufficiently powered to find statistically significant associations in the individual cohorts. Therefore, the effect sizes and standard errors of the individual analyses were combined in an inverse-weighted variance meta-analysis to test the overall association. This was allowed because the obtained effect sizes of the individual datasets, although different methods were used to score joint destruction (SHS and Larsen), all represented the relative increase (without units) of progression in joint destruction per year. The meta-analysis weights the results with a low standard error stronger than the results with a high standard error, preventing an overrepresentation of less precise data on the outcome. Subsequently, datasets with smaller 95% confidence intervals (CI) had a larger weight in the meta-analysis.

The cut-off for statistical significance was set at $p<4.17\times10^{-3}$ using the Bonferroni correction (four variants tested in the total RA population and ACPA-positive and ACPA-negative subgroups: 0.05/12 tests=4.17×10^{-3}). For the fine-mapping analyses the cut-off for statistical significance was set at $p<1.18\times10^{-4}$, also using the Bonferroni correction (0.05/424 tests=1.18×10^{-4}). Analyses were performed using IBM SPSS version 20 and Stata version 12.0.

RESULTS

Patient characteristics and analyses on total RA population

The minor allele frequencies for rs1800795 (G) in *IL-6*, rs1800896 (T) in *IL-10*, rs2900180 (A) in *C5-TRAF1* and rs3761959 (A) (=perfect proxy rs7528684 (G)) in *FCRL3* in the different cohorts are presented in Table 1. First, analyses were done on the total RA population. Statistical significance was obtained in none of the individual cohorts. The directionality of the effects was variable across the cohorts (Figure 1). Also in the meta-analyses on the six cohorts (2,493 patients and 5,895 sets of radiographs in total) no significant associations were obtained for rs1800795 in *IL-6* (fixed effects model p=0.72), rs1800896 in *IL-10* (fixed effects model p=0.93), rs2900180 in *C5-TRAF1* (fixed effects model p=0.22) and rs7528684 in *FCRL3* (fixed effects model p=0.83).

Analyses of ACPA-positive and ACPA-negative RA

As some of the initial reports on these four genetic variants stratified or adjusted the analyses

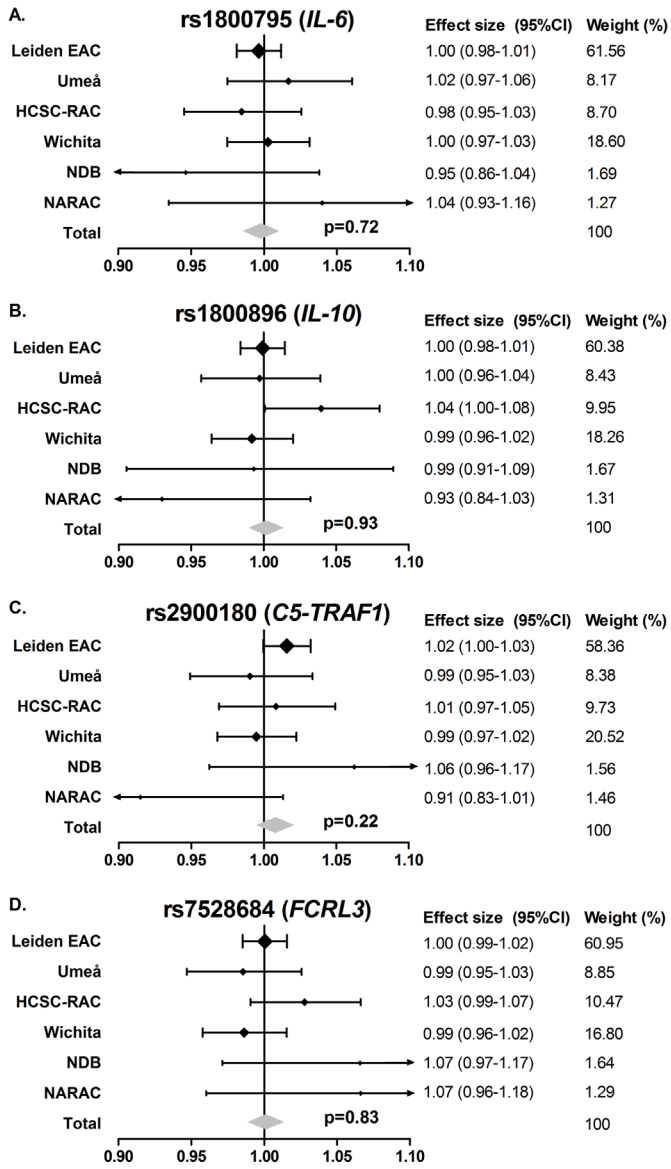

Figure 1. Genetic variants in *IL-6* (A), *IL-10* (B), *C5-TRAF1* (C) and *FCRL3* (D) in relation to radiographic joint damage progression. Presented are the yearly radiographic progression rates per individual cohort and the meta-analyses evaluating the six cohorts combined, consisting in total of 2,493 patients and 5,895 sets of radiographs. None of the studied genetic variants were significantly associated with radiographic progression, neither in the individual cohorts nor in meta-analysis. Rs1800795 (*IL-6*) I^2 0.0%, p=0.67; fixed effect p=0.72, random effect p=0.72; rs1800896 (*IL-10*) I^2 20.8%, p=0.28; fixed effect p=0.93, random effect p=0.89; rs2900180 (*C5-TRAF1*) I^2 28.7%, p=0.22; fixed effect p=0.22, random effect p=0.63; rs7528684 (*FCRL3*) I^2 26.0%, p=0.24; fixed effect p=0.83, random effect p=0.73.

for the presence of ACPA and as ACPA-positive and ACPA-negative RA are considered as separate disease entities, analyses were performed on radiographic progression in ACPA-positive and ACPA-negative RA separately. The ACPA-positive subgroup comprised 1,748 patients (with 3,820 sets of radiographs) who were included in six cohorts. The ACPA-negative subgroup included 681 patients (with 1,933 sets of radiographs) who were included in the EAC, Umeå, HCSC-RAC and NDB cohorts (Table 1).

Rs1800795 (*IL-6*), rs1800896 (*IL-10*), and rs7528684 (*FCRL3*) were not associated with radiographic progression, neither in the ACPA-positive nor in the ACPA-negative group of RA patients (Additional file 2). Rs2900180 in *C5-TRAF1* was not associated with radiographic progression in ACPA-positive RA (Additional file 2). In contrast, in ACPA-negative RA a significant association with radiographic progression was observed in the EAC ($p=2.88\times10^{-5}$) (Figure 2A). The directionality of the effect was similar in the Umeå, HCSC-RAC and NDB cohorts. Also, the meta-analysis revealed a significant association (fixed effects model $p=5.85\times10^{-7}$) (Figure 2B). In all cohorts, patients with the minor allele had a higher rate of joint destruction. For instance, RA patients included in the EAC with one minor allele had a 1.045 fold rate of joint destruction per year compared to patients with the common genotype; this equals a 36% (1.045^7) higher rate of joint destruction over seven years (Figure 2A).

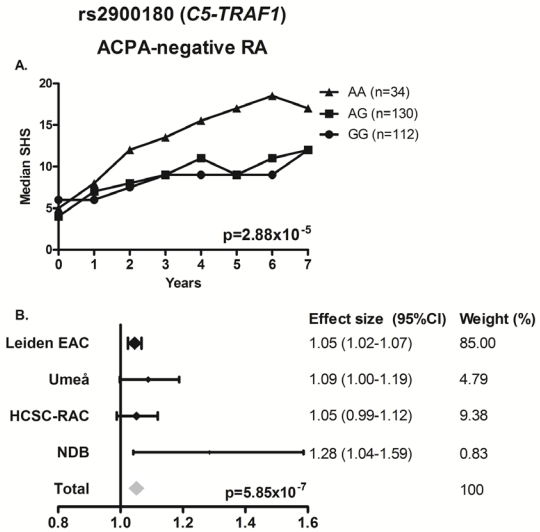

Figure 2. Rs2900180 in *C5-TRAF1* in relation to radiographic progression in ACPA-negative RA patients. (A) Depicted are the median SHSs during seven years of follow-up of ACPA-negative RA patients with different genotypes in the Leiden EAC. Patients had per minor allele a 1.045 fold rate of joint destruction per year compared to patients with the common genotype ($p=2.88\times10^{-5}$). (B) Yearly radiographic progression rates per individual cohort and the meta-analysis evaluating the cohorts with ACPA-negative patients. Analysis of the ACPA-negative subgroup of the Wichita cohort was not performed as it included only three ACPA-negative patients. I^2 33.0%, p= 0.22; fixed effect $p=5.85\times10^{-7}$, random effect p=0.0024.

Fine-mapping

To examine if other genetic variants within the *C5-TRAF1* region had statistically stronger associations with the rate of joint destruction than rs2900180, this region was fine-mapped in the ACPA-negative EAC patients. In total, 43 variants had a p-value below the threshold for multiple correction ($p<1.18\times10^{-4}$) of which 34 were statistically more strongly associated with radiographic progression than rs2900180 (Figure 3, Additional file 3). The 43 associating variants, including rs2900180, were all located within a 66 Kb region spanning *TRAF1* and extending downstream to the *C5-TRAF1* intergenic region and upstream to the *TRAF1*-PHF19 intergenic region. The variant with the lowest p-value was rs7021880 located in *TRAF1* (beta=1.052 per year per minor allele, $p=1.39\times10^{-6}$). In a conditional analysis on rs2900180 and rs7021880 ($R^2=0.864$), both variants lost statistical significance (rs2900180 beta=0.99 p=0.77; rs7021880 beta=1.06 p=0.057). This suggests that these two variants reflected one signal, although it is noteworthy that the effect size of rs2900180 was reduced to 0.99 and the effect size of rs7021880 increased slightly. Additionally, the fine-mapping analyses were performed when conditioning on the strongest associating variant rs7021880. No variants were statistically significant associated with radiographic progression independent of rs7021880 (Additional file 4). In meta-analysis of the ACPA-negative patients of the EAC, Umeå, HCSC-RAC and NDB cohorts rs7021880 was significantly associated with radiographic progression (fixed effects model $p=6.35\times10^{-8}$) (Additional file 5).

Downstream effect

To identify functional downstream effects of the region of rs7021880, a database and literature search of transcription studies was performed. The RegulomeDB indicated that this locus has multiple signs of transcriptional activity [21]. Based on RNA expression evaluated by eQTL mapping of peripheral blood samples of 8,086 individuals [22], the minor allele of rs7021880 was negatively correlated with RNA expression of different genes in this region (cis-eQTL), with the lowest p-value for the expression of *TRAF1* ($p=4.93\times10^{-35}$) (Additional file 6). However, the strongest correlation between variants in this region with *TRAF1* expression was observed for rs2416804 in *TRAF1* (D'=1.000, $R^2=0.668$ with rs7021880). A study evaluating CD4+ T-cells and monocytes of 461 individuals observed that several variants in the region of rs7021880 had cis-eQTL effects on *TRAF1* (in T-cells) and on *C5* (in monocytes) [23]. Both studies explored constitutive expression [22,23]. The effect of a regulatory variant on gene expression, however, may depend on the presence of certain stimuli. Response eQTLs have been studied in different cell types using different stimuli [24–26]. In lymphoblast cell lines of 40 Asian individuals variants in the *C5-TRAF1* region were associated with *TRAF1* expression after phorbol myristate actetate (PMA) stimulation, but not with *C5* expression [24]. Monocytes are cardinal innate immune cells that upon stimulation, exhibit large scale gene transcription and cytokine production. A recent study in 432 individuals showed that the expression of *TRAF1* and *C5* significantly changed in monocytes after stimulation with lipopolysaccharide (LPS) [25]. Furthermore, rs7021880, associated with radiographic progression in our study, as

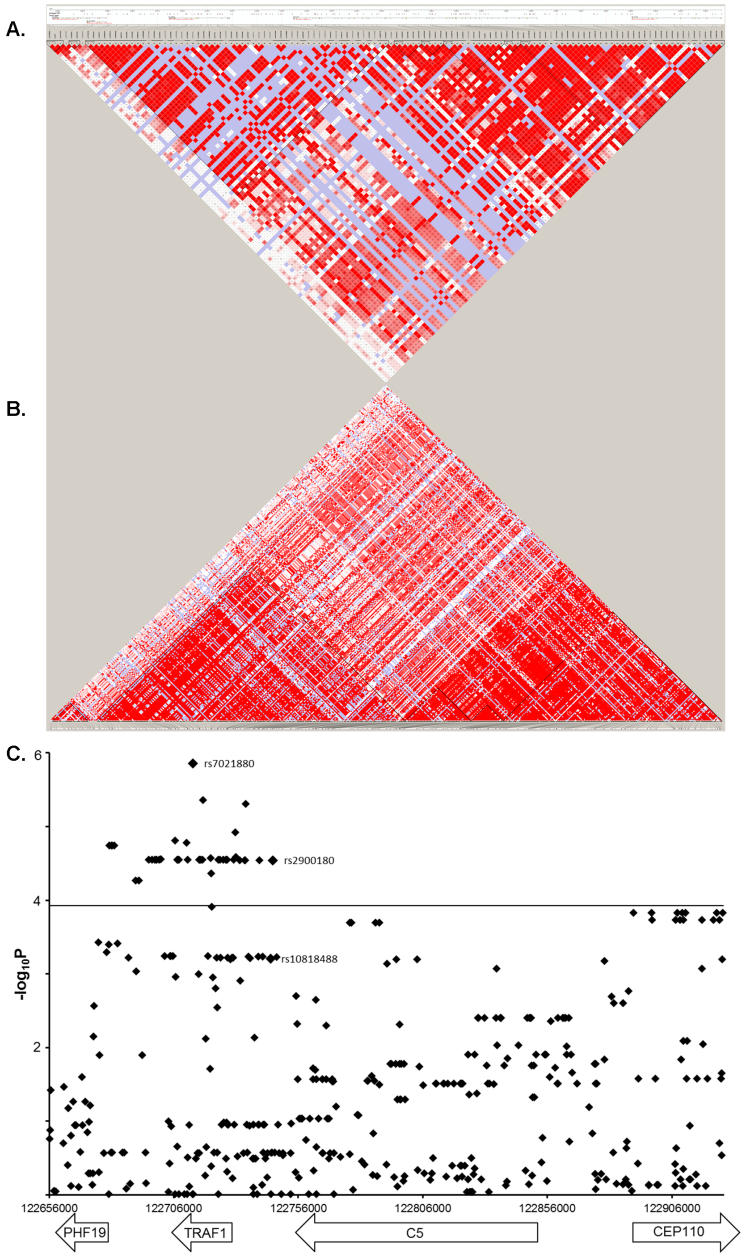

Figure 3. LD plots of the *C5-TRAF1* region. (A) Total region in hapmap CEU patients and (B) fine-mapped in Leiden EAC. The colors reflect de D' between the SNPs. Coordinates relate to NCBI36 hg18 release 2006. (C) Results of the multivariate normal regression analysis for 424 variants in the *C5-TRAF1* region in the ACPA-negative patients of the EAC. Rs2900180 is the initially studied variant, rs7021880 is the variant with the lowest p-value and rs10818488 is the variant we previously studied in relation to radiographic progression and did not associate with radiographic progression in the total population [29]. Also in the current study, rs10818488 did not pass the cut-off for multiple testing correction in the ACPA-negative patients. Using the Bonferroni correction (considering 424 variants studied) the cut-off for statistical significance was set at 1.18×10^{-4} as represented by the horizontal line.

well as several other variants in this region, affected gene expression after two hours of LPS stimulation; a strong cis-effect was seen for expression of *TRAF1* (p for rs7021880 = 1.20×10^{-6}, t-stat=−5.00) (Additional file 7) [25]. Similarly, in a comparable study of stimulation-specific eQTLs in dendritic cells derived from peripheral blood monocytes of 534 individuals, the RA susceptible variant rs881375 in the intergenic *TRAF1*-PHF19 region (R^2=0.902, D'=1.000 with rs7021880) showed response eQTL after LPS and influenza stimulation (respectively $p=6.33\times10^{-8}$ and 1.04×10^{-10}) [26]. Together these data indicate a response eQTL effect on monocytes and dendritic cells derived from monocytes for rs7021880 and its proxy SNPs.

DISCUSSION

This study aimed to increase the understanding of the relevance of four previously identified risk factors for the severity of joint destruction in RA. To this end, 2,493 RA patients (and 5,895 sets of radiographs) included in six independent cohorts from different parts of Europe and North America were studied. In contrast to previous observations in smaller studies, the variants in *IL-6*, *IL-10* and *FCRL3* were not associated with radiographic progression. This indicates that these variants do not mediate the severity of structural damage in RA. A statistically significant association, confined to the ACPA-negative subgroup of RA was found for rs2900180 in *C5-TRAF1*. Fine-mapping of this region revealed the lowest p-value for rs7021880 in *TRAF1*, although this signal was not independent of other variants in this region. The putative relevance of rs7021880 and its surrounding region was supported by differences in RNA expression of *TRAF1* in peripheral blood and monocytes in relation to these genotypes.

We have strongly considered whether the findings on the *C5-TRAF1* region may be false positive. However, despite the fact that all replication cohorts individually had less power than our first cohort and it was unlikely to find statistically significant results in the individual replication cohorts, the obtained effect sizes and directionalities were similar in the Umeå, HCSC-RAC and NDB cohorts. Also, the meta-analysis on these cohorts was highly significant ($p=6.35\times10^{-8}$ for rs7021880). Therefore, in our view, it is more likely that the finding on *C5-TRAF1* in ACPA-negative RA is a true positive finding than a false positive finding.

The association of rs2900180 in *C5-TRAF1* with radiographic progression was previously observed in two studies on the total RA population [8,9]. The first study concerned 761 RA patients of the Norfolk Arthritis Register (NOAR) with a radiograph after one or five years; 61% of these patients were ACPA-negative. In ACPA-stratified analyses after one year follow-up the effect was significant in ACPA-negative RA patients but not in ACPA-positive RA patients [8]. The second study reevaluated the NOAR with longitudinal radiographs and also included patients of the Early Rheumatoid Arthritis Study (ERAS); ACPA status was not reported here [9]. The difference in findings in the UK cohorts and our cohorts with regard to ACPA might be the consequence of different frequencies of ACPA-negativity between the

cohorts. In the NOAR, the proportion of ACPA-negative patients was higher than in our cohorts (61% versus 28% of the total study population of the six cohorts) [8]. Therefore, the ACPA-negative patients may have contributed more to the results obtained for the NOAR total RA population than in the total RA populations studied here. The consistency of the directionality of the effect (the minor allele associated with more severe damage) in the cohorts studied in the present study and the previously studied cohorts of NOAR and ERAS supported the validity of our findings.

Fine-mapping was performed to explore the *C5-TRAF1* region comprising rs2900180 which is located on chromosome 9 and intergenic between *C5* and *TRAF1*. The threshold for significance of the fine-mapping analyses was corrected for 424 tests which is quite restrictive as the markers included in the analyses are not independent. However, this threshold decreased the chance of false positive findings and, in total, 43 variants had a p-value below this threshold. These variants were all highly correlated and located in a 66 Kb region covering *TRAF1* and downstream extending to the *C5-TRAF1* intergenic region and upstream to the *TRAF1*-PHF19 intergenic region. Also, these 66 Kb are located within a larger region with a high LD spanning from *C5* to *PHF19* (based on Ceu HapMap data and as described previously [28]). In a conditional analysis including both the initial (rs2900180) and the strongest associating variant (rs7021880), we could not distinguish which variant is the most important. Therefore, the conclusion is that the region encompassing rs7021880 and rs2900180 is associated with radiographic progression. Larger fine-mapping studies are required to conclude definitely on the extent of the region that presumably contains the causal variant.

Previously, we reported that rs10818488, which is also located in the *C5-TRAF1* region (intergenic *C5-TRAF1*), was not associated with radiographic progression in 2,666 RA patients belonging to seven cohorts. No stratification for ACPA was done for this analysis [29]. Also, when rs18018488 was analysed in the total RA population of the present study which included four cohorts that were studied previously and two additional cohorts, no significant associations were obtained (data not shown). In the present study, rs10818488 was also included in the fine-mapping data of the ACPA-negative patients and did not pass the threshold for multiple testing (which was $p<1.18\times10^{-4}$; the p-value for rs10818488 was 6.21×10^{-4}) (Figure 3C). To explore the relation between rs10818488 and rs2900180 genotypes, the genotypes were compared (Table 2), showing incomplete correlations which is in line with the R^2 of 0.668 between these two variants. For instance, all patients with genotype AA for rs2900180 had genotype AA for rs10818488, but also other patients had genotype AA for rs18018488. In total, 41 of the 276 ACPA-negative patients (14.9%) had different genotypes which explains the difference in the obtained p-values for rs10818488 and rs2900180. The minor allele of rs2900180 that associated in the present study with a higher rate of joint destruction in ACPA-negative RA is also associated with a higher risk of RA [18,28]. Rs2900180 was observed to associate with susceptibility to RA in both Caucasian and Korean

Table 2. Genotypes of rs10818488 and rs2900180 in the ACPA-negative Leiden EAC patients

		rs2900180 (A)			Total
		GG	AG	AA	
rs10818488 (A)	GG	88	0	0	88
	AG	22	113	0	135
	AA	2	17	34	53
Total		112	130	34	276

Presented are the frequencies of the genotypes for rs2900180 (A) and rs10818488 (A) in the 276 ACPA-negative RA-patients of the Leiden EAC. The R^2 between these variants was 0.668. Minor allele frequencies within this group were 35.9% and 43.7% for rs2900180 and rs10818488, respectively.

patients, in contrast to rs10818488, which was observed to be a risk factor for RA only in Caucasian patients [30]. Hence, apparently not only the association with RA severity but also the associations with RA susceptibility are slightly different for rs2900180 and rs10818488.

A correlation of rs7021880 located in *TRAF1* with *TRAF1* expression was observed in whole blood, although rs7021880 was not the strongest associating genetic variant with *TRAF1* expression [22]. These data are valuable but reflect on a mixture of cells and constitutive expression. Interestingly, very recently two studies evaluated eQTL effects on RNA expression of monocytes or dendritic cells derived from monocytes after several stimuli (response eQTL). These data are attractive since monocytes play a relevant role in the development and progression of RA and because it is conceivable to suggest that variants that associate with progression of the disease are expressed in response to inflammatory stimuli. Hence, differences in such expression may affect the disease course. The expression of *TRAF1* in monocytes was significantly altered after LPS stimulation compared to naïve monocytes and rs7021880 genotypes were associated with this change in expression after stimulation [25]. Genetic variants might thus affect the level of *TRAF1* expression in response to stimulation. Similar findings were observed in dendritic cells derived from monocytes for rs881375, a good proxy of rs7021880 (R^2=0.902) [26]. *TRAF1* is involved in the NF-κB pathway, providing a potential pathway for how these genetic variants may influence progression of joint destruction. The analyses on the large bioinformatics databases supported the notion that the region surrounding rs7021880 has a regulatory function in monocytes, but these databases did not allow us to perform conditional analyses on the genetic variants in this region in relation to RNA expression to identify independent effects. In addition, in several of the studies explored the directionality of the effects on expression was not clearly presented, hampering the interpretation of the potential effects of rs7021880. Although most studies reported on the expression of *TRAF1*, eQTL effects on *C5* in certain cell types have also been reported. The data available do not allow us to conclude whether effects on expression are consistent across cell types. More studies are needed to explain how the *C5-TRAF1* region is relevant for radiographic progression in ACPA-negative RA.

The variants in *IL-6*, *IL-10* and *FCRL3* were not associated with radiographic

Table 3. Overview of genetic variants for radiographic progression that are replicated in independent cohorts or found significant in meta-analysis

Severity variant (risk allele)	Located in/nearby gene(s) (chromosome)	Risk population	Functional associations
SE [31]	*HLA-DRB1* (chr 6)	All RA	Associated with ACPA-presence
rs4810485 (T) [32]	*CD40* (chr 20)	ACPA-pos	NA
rs7667746 (G) [33]	*IL-15* (chr 4)	All RA*	NA
rs7665842 (G) [33]			
rs4371699 (A) [33]			
rs6821171 (A) [33]			
rs1896368 (G) [34]	*DKK-1* (chr 10)	All RA	Serum level DKK-1
rs1896367 (G) [34]			
rs1528873 (A) [34]			
rs2104286 (T) [35]	*IL2RA* (chr 10)	All RA	Serum level IL2Rα
rs8192916 (A) [36]	*GRZB* (chr 14)	All RA[+]	RNA expression in whole blood (eQTL)
rs1119132 (A) [37]	*IL4R* (chr 16)	All RA[§]	NA
rs7607479 (T) [38]	*SPAG16* (chr 2)	ACPA-pos	Serum level MMP-3
rs26232 (C) [39]	*C5orf30* (chr 5)	All RA**	NA
rs11908352 (A) [20]	*MMP-9* (chr 20)	All RA[++]	Serum level MMP-9
rs451066 (A) [20]	*rs1465788* (chr 14)	All RA[§§]	NA
rs1485305 (G) [40]	*OPG* (chr 8)	All RA***	NA
rs2900180 (A)	*C5-TRAF1* (chr 9)	ACPA-neg	RNA expression whole blood and monocytes

*After adjustment for ACPA, comparable effect sizes were observed (data not shown). [+]After stratification for ACPA, significant associations were observed in both subgroups (ACPA-negative beta=1.05 and p=1.98x10^{-3}; ACPA-positive beta=1.03 and p=5.40x10^{-2}). [§]After stratification for ACPA, comparable effect sizes were observed in both subgroups (data not shown). **After adjustment for ACPA and RF a significant association was observed (beta=0.90, p=0.03). [++] After stratification for ACPA, the effect size was larger in the ACPA-positive than in the ACPA-negative subgroup. However, considering the small number of patients per subgroup, none of the analyses resulted in significant p-values. [§§]After stratification for ACPA, almost similar effect sizes were observed in both subgroups. However, considering the small number of patients per subgroup, none of the analyses resulted in significant p-values. ***After stratification, a significant association was observed in ACPA-negative patients (beta=1.29, p=0.001) but not in ACPA-positive patients, although a similar trend was observed (beta=1.14, p=0.11). After adjustment for ACPA and RF the association remained significant (beta=1.20, p=0.02). eQTL=expression quantitative trait locus; NA=not applicable

progression. Also, the directions of the effects between the different cohorts were diverse and no tendency for association was observed. The initial findings on these variants were obtained in studies with a lower number of patients and radiographs than in present study.

This study was started in response to findings of a recent review of the literature on genetic variants that are associated with radiographic progression in RA [4]. Although genotyping data of five of the cohorts were retrieved from the Immunochip, we did not intend to analyse the whole Immunochip, as this was done recently in a study that

included three of the six studies that are examined in the present study [20]. This study was focused on the variants for which existing data were contradictory and we went into detail by also performing analyses stratified for ACPA. The total number of genetic variants for radiographic progression that are identified and either replicated in independent cohort studies or found significant in meta-analysis is now thirteen (including *C5-TRAF1*) of which nine were identified in the total RA population (summarised in Table 3). The ACPA-negative subgroup was studied separately in only five studies (excluding the present study). Of these, rs8192916 in *GRZB* and rs1485305 in *OPG* were reported to have a statistically significant association with radiographic progression within the ACPA-negative subgroup; for the other three variants (rs1119132 in *IL4R*, rs11908352 in *MMP-9* and rs451066 on chromosome 14) similarity in effect sizes were reported but statistical significance was not obtained which may be due to smaller sample sizes. The data in Table 3 and the present data show that genetic risk factors for radiographic progression in ACPA-positive and ACPA-negative RA are not similar and further support the notion of two separate disease subsets of RA.

CONCLUSION

In conclusion, in contrast to initial reports, variants in *IL-6*, *IL-10* and *FCRL3* are not associated with radiographic progression. The association between rs2900180 in *C5-TRAF1* and radiographic progression is confined to ACPA-negative RA. A region surrounding rs2900180 affects *TRAF1* expression in whole blood and monocytes. Further functional studies are needed to elucidate the underlying biological mechanisms in more detail.

SUPPLEMENTARY DATA

Supplementary data are published on the website of *Arthritis, Research & Therapy*.

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Association of Valine and Leucine at HLA-DRB1 position 11 with radiographic progression in rheumatoid arthritis, independent of the shared epitope alleles but not independent of anti-citrullinated peptide antibodies

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ABSTRACT

Objective

For decades it has been known that the HLA-DRB1 shared epitope (SE) alleles are associated with an increased risk of development and progression of rheumatoid arthritis (RA). Recently, the following variations in the peptide-binding grooves of HLA molecules that predispose to RA development have been identified: Val and Leu at HLA-DRB1 position 11, Asp at HLA-B position 9, and Phe at HLA-DPB1 position 9. This study was undertaken to investigate whether these variants are also associated with radiographic progression in RA, independent of SE and anti-citrullinated peptide antibody (ACPA) status.

Methods

A total of 4,911 radiograph sets from 1,878 RA patients included in the Leiden Early Arthritis Clinic (the Netherlands), Umeå (Sweden), Hospital Clinico San Carlos-Rheumatoid Arthritis (Spain), and National Data Bank for Rheumatic Diseases (US) cohorts were studied. HLA was imputed using single-nucleotide polymorphism data from an Immunochip, and the amino acids listed above were tested in relation to radiographic progression per cohort using an additive model. Results from the 4 cohorts were combined in inverse-variance weighted meta-analyses using a fixed-effects model. Analyses were conditioned on SE and ACPA status.

Results

Val and Leu at HLA-DRB1 position 11 were associated with more radiographic progression (meta-analysis $p=5.11 \times 10^{-7}$); this effect was independent of SE status (meta-analysis p=0.022) but not independent of ACPA status. Phe at HLA-DPB1 position 9 was associated with more severe radiographic progression (meta-analysis p=0.024), though not independent of SE status. Asp at HLA-B position 9 was not associated with radiographic progression.

Conclusion

Val and Leu at HLA-DRB1 position 11 conferred a risk of a higher rate of radiographic progression independent of SE status but not independent of ACPA status. These findings support the relevance of these amino acids at position 11.

INTRODUCTION

The development and course of rheumatoid arthritis (RA) are in part determined by genetic factors. Although the genetic risk factors underlying RA development and progression of joint destruction are largely non-overlapping [1], the genetic variants encoding the so-called HLA-DRB1 shared epitope (SE) alleles are associated with both the risk of RA development and the severity of the disease course [2–4].

The association of HLA class II with RA has been known for decades. The association between HLA-DR and RA was first reported in 1976 [5]. Subsequent identification of risk HLA-DRB1 alleles that all shared a similar amino acid sequence at positions 70–74 in the peptide-binding groove of the HLA-DRB1 molecule led to the formulation of the SE hypothesis [6]. This hypothesis postulates that the SE motif itself may be directly involved in the pathogenesis of RA by allowing the presentation of an arthritogenic peptide to T cells. Thus far, these peptides have not been identified. With the identification of anti-citrullinated peptide antibodies (ACPA) in the late 1990s, it became clear that SE alleles mainly predispose to ACPA-positive RA [3,7]. The relevance of HLA-DRB1 for ACPA-negative RA was set by the identification of HLA-DRB1*03 (part of the conserved ancestral A1-B8-DRB1*03 haplotype) as a risk factor for ACPA-negative RA [8,9].

Recently, a further refinement of the association between HLA and RA was proposed by Raychaudhuri et al [10]. Using a case-control design with 5,018 ACPA-positive RA patients and 14,974 controls, the class I and class II HLA regions were explored. The strongest association was reported for HLA-DRB1 positions 11 and 13 (which are in high linkage disequilibrium). The amino acids Val and Leu at position 11 conferred a high risk, and Ser was protective. These associations were independent of the SE status. Furthermore, performing further conditional analyses, independent associations were observed for variants in HLA-B position 9 (Asp predisposed to RA) and HLA-DPB1 position 9 (Phe predisposed to RA). In a subsequent study using a similar approach, the authors also investigated 2,406 ACPA-negative RA patients and 13,930 controls [11] and observed that Leu and Ser at HLA-DRB1 position 11 and Asp in HLA-B position 9 were associated with an increased risk of ACPA-negative RA.

These risk positions are located in the peptide-binding grooves of the HLA molecules. Studies of MHC class I and class II in mice have shown that a difference of only one or a few amino acids at such a crucial place may result in the presentation of totally different peptides [12,13]. Therefore, the finding that additional amino acids located in the antigen-presenting binding grooves associate with RA development is relevant and hypothetically may fuel further studies to detect arthritogenic peptides involved in RA susceptibility [10]. Because the HLA-DRB1 SE alleles are among the strongest genetic factors for a progressive disease course, the recent findings of Raychaudhuri et al prompted us to determine the relevance of the newly identified risk factors for the severity of the course of RA, measured using radiographic

joint damage progression. More specifically, first, we aimed to investigate whether Val, Leu, and Ser at HLA-DRB1 position 11, Asp at HLA-B position 9, and Phe at HLA-DPB1 position 9 are associated with radiographic progression in the total RA population and, if so, whether these effects are independent of the well-known SE effect (HLA-DRB1 positions 70–74). Second, since the SE alleles predispose to ACPA and the SE alleles are not associated with radiographic progression independent of ACPA [7,14], we aimed to analyse whether the newly identified associations are independent of ACPA. Third, we aimed to evaluate whether Leu and Ser at HLA-DRB1 position 11 and Asp at HLA-B position 9, identified as risk factors for ACPA-negative RA, are associated with joint damage progression in ACPA-negative RA. To this end, a total of 4,911 sets of radiographs of 1,878 patients with RA in 4 different cohorts were studied.

PATIENTS AND METHODS

Patients

Patients were included from the following 4 cohorts: the Leiden Early Arthritis Clinic (EAC), the Umeå cohort, the Hospital Clinico San Carlos-Rheumatoid Arthritis Cohort (HCSC-RAC), and the National Data Bank for Rheumatic Diseases (NDB). In all cohorts, RA was defined according to the American College of Rheumatology 1987 criteria [15]. The characteristics of the patients in each cohort are presented in Supplementary Table 1. Informed consent was obtained from all patients, and approval was obtained from the local ethics committee of each study.

Leiden EAC - This cohort consisted of 594 Dutch patients with early RA who were enrolled between 1993 and 2006 [16]. The mean±SD age at diagnosis was 57.0±15.6 years, 67.0% of the patients were women, and 52.8% were ACPA-positive (as determined by anti-cyclic citrullinated peptide 2 [anti-CCP2] antibody test). Radiographs of the hands and feet were obtained at baseline and during yearly follow-up visits. In total 3,121 sets of radiographs obtained over 7 years of follow-up were chronologically scored by 1 experienced reader, who was blinded with regard to any clinical or genetic data, using the Sharp-van der Heijde score (SHS) (within reader intraclass correlation coefficient [ICC] 0.91). Initial treatment strategies changed over time. Patients enrolled in 1993-1995 were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs), patients enrolled in 1996-1998 were initially treated with hydroxychloroquine or sulfasalazine, and patients enrolled in 1999-2006 were initially treated with methotrexate.

Umeå cohort - This Swedish cohort comprised 365 patients with early RA enrolled between 1995 and 2010. The mean±SD age at diagnosis was 54.3±14.5 years, 69.6% of the patients were women, and 71.8% were ACPA-positive (as determined by anti-CCP2 antibody test). At baseline and after 2 years, a total of 687 radiographs of the hands and feet were obtained and scored using the Larsen score by 2 trained readers as previously described [17]. Treatment

strategies differed between 1995-2000, 2000-2005, and 2006-2010, resulting in less severe radiographic progression in the subsequent treatment periods.

HCSC-RAC - This Spanish dataset involved 380 patients with early RA, diagnosed between 1976 and 2011 [18]. The mean±SD age at diagnosis was 53.8±14.2 years, 76.3% of the patients were women, and 48.6% were ACPA-positive (as determined by anti-CCP2 antibody test). During the first 10 years after disease onset, 564 radiographs of the hands were obtained and scored with known time-order using the SHS (ICC 0.99). Initial treatment strategies differed for different inclusion periods. Prior to 1990, patients were initially treated with NSAIDs, from 1990 to 1999 patients received initial monotherapy with conventional disease-modifying antirheumatic drugs, from 2000 to 2004 patients received initial monotherapy regularly and combination therapy rarely, from 2005 to 2009 patients received initial combination therapy regularly as well as biologic agents, and from 2010 to 2011 patients received tailored treatment.

NDB - This dataset included 539 patients from the US and Canada who were diagnosed between 1980 and 1999 [19]. The mean±SD age at diagnosis was 48.6±12.6 years, 78.5% of the patients were women, and 79.6% were ACPA-positive (as determined by anti-CCP2 antibody test). One set of hand radiographs was available per patient after a mean disease duration of 10.1 years (range 1–20 years) and scored according to the SHS (ICC 0.98). No treatment effects were observed for different periods of diagnosis since all patients developed RA in eras when early, tailored treatment and the use of biologic agents were uncommon.

Genotyping

For all cohorts, we used SNP2HLA to impute classic alleles and corresponding amino acid polymorphisms for HLA class I loci (HLA-A, -B, and -C) and class II loci (HLA-DPA1, -DPB1, -DQA1, -DQB1, and -DRB1) [20]. We obtained patient genotypes using an Illumina Immunochip platform [1,21] and used data collected by the Type 1 Diabetes Genetics Consortium as a reference [22]. The HLA imputation has been described in detail previously [10,11,20]. For the present study, the classic HLA-DRB1 alleles and the amino acids at HLA-DRB1 position 11, HLA-B position 9, and HLA-DPB1 position 9 were extracted.

Analyses of the associations between variants and radiographic progression

First, the SE alleles, defined based on HLA-DRB1 positions 70-74 according to the SE hypothesis [6] (DRB1*0101, *0102, *0104, *0401, *0404, *0405, *0408, *0413, *0416, *1001, and *1402), were tested in relation to radiographic progression. Subsequently, the amino acids that were shown to confer risk to develop RA in the study by Raychaudhuri et al [10] (Val and Leu at HLA-DRB1 position 11, Asp at HLA-B position 9, and Phe at HLA-DPB1 position 9) were studied in relation to radiographic progression. Analyses were done per cohort using an additive model (0, 1, or 2 risk amino acids). Since Val and Leu at position 11 both conferred increased risk, these were combined (i.e., radiographic progression in the presence of 0, 1, or 2 Val and/or Leu was studied). Because the SE alleles and Val or Leu on position 11 are often

present together, conditional analyses were performed allowing us to investigate whether Val and Leu were associated with radiographic progression independent of the known association between HLA-DRB1 SE status and radiographic progression. Furthermore, for the SE alleles it is known that the alleles are not associated with radiographic progression independent of ACPA; in other words, the SE alleles are not associated with radiographic progression as such but this effect is mediated by ACPA [7,14]. To determine whether the same is valid for the newly identified risk factors, analyses were subsequently adjusted for ACPA status.

Previously, the amino acid Ser at HLA-DRB1 position 11 was found to protect against RA development [10]. In this study, the association of Ser (0, 1, or 2 amino acids) with radiographic progression was analysed.

The above-mentioned analyses of Val and Leu and of Ser at HLA-DRB1 position 11 do not take into account the polymorphic nature of HLA-DRB1 in which different amino acids encoded by the same position may have a predisposing or protective effect. It is crucial to ascertain that the predisposing effect identified is not actually due to the absence of a protective effect and vice versa. Stratification is the most thorough method to differentiate between these effects and has been used before to distinguish true effects from the effect of the absence of other alleles [2,23,24]. Therefore, patients were stratified into one of the following 6 groups: susceptible/ susceptible (S/S), susceptible/neutral (S/N), susceptible/ protective (S/P), neutral/neutral (N/N), neutral/protective (N/P), and protective/protective (P/P). Val and Leu at HLA-DRB1 position 11 were the susceptibility amino acids, Ser at this position was the protective amino acid, and Asp, Pro, and Gly were the neutral amino acids. Then the groups of S/S, S/N, and N/N were compared to determine whether the effect of Val and Leu was truly predisposing and not the result of the concomitant absence of Ser. Similarly, the groups of P/P, P/N, and N/N were compared to determine whether the effect of Ser was truly protective and not the result of the concomitant absence of the susceptibility amino acids Val and Leu.

Finally, we evaluated whether the variants that were reported to predispose to ACPA-negative RA [11] (Leu and Ser at HLA-DRB1 position 11 and Asp at HLA-B position 9) were associated with radiographic progression in ACPA-negative RA.

Statistical analysis

In all datasets, radiographic scores were log-transformed (log(score+1)) to approximate a normal distribution. The residuals of the used models were normally distributed around the 0-line in all cohorts, indicating a good fit of the models. In the cohorts in which multiple sets of radiographs were obtained over time (EAC, Umeå, and HCSC-RAC), a multivariate normal regression model for longitudinal data was used with radiographic scores as outcome [1,25,26]. This method takes advantage of the within-person correlation between repeated measurements; as such the radiographic progression rates were estimated more precisely in the cohorts with serial radiographs compared to datasets with one radiograph per patient (for a detailed description, see ref. 26). In the cohort in which a radiograph set was obtained at

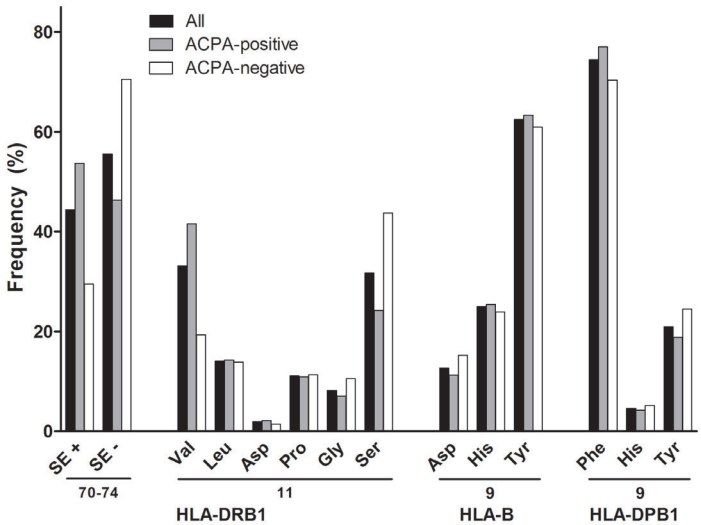

Figure 1. Frequencies of HLA-DRB1 shared epitope (SE) alleles and the amino acids at HLA-DRB1 position 11, HLA-B position 9, and HLA-DPB1 position 9. Frequencies are expressed as the percentage of a total of 3,756 alleles/amino acids. Of the 1,878 patients, 69.0% were positive for the HLA-DRB1 SE (at least 1 SE allele), 71.2% were positive for Val/Leu at HLA-DRB1 position 11 (at least 1 Val or Leu amino acid), 23.8% were positive for Asp at HLA-B position 9 (at least 1 Asp), and 93.0% were positive for Phe at HLA-DPB1 position 9 (at least 1 Phe).

a single time point (NDB), the estimated yearly progression rate was calculated (total SHS/disease duration in years at time of radiograph) to make the estimates of the progression rates comparable to those in the other datasets. Linear regression analysis was used with estimated yearly progression as outcome variable. In both models used, the effect sizes obtained were back-transformed and indicated the fold rate of joint destruction per year per risk amino acid compared to the reference.

In all datasets, adjustments were made for age and sex. In the cohorts that included patients in periods with different treatment strategies (EAC, Umeå, and HCSC-RAC), analyses were also adjusted for the inclusion period as proxies for differences in treatment strategies.

The individual datasets studied were estimated to be insufficiently powered to find statistically significant associations when performing conditional analyses. Therefore, the effect sizes and standard errors of the results from the individual cohorts were combined in inverse-weighted variance meta-analyses with fixed effects to test the overall association. This was allowed because the effect sizes obtained for the individual datasets, though different methods were used to score joint destruction (SHS and Larsen), all represented the relative increase (without units) of progression in joint destruction per year. The meta-analysis weights the results with a low standard error stronger than the results with a high standard error, preventing an overrepresentation of less precise data in the outcome. Subsequently, datasets with smaller 95% confidence intervals had a larger weight in the meta-analysis.

P-values less than 0.05 were considered significant. Statistical analysis was performed using SPSS version 20 and Stata 12.0.

RESULTS

Frequencies of variants

The allele frequencies of HLA-DRB1 SE and the amino acids at HLA-DRB1 position 11, HLA-B position 9, and HLA-DPB1 position 9 in the total population studied are presented in Figure 1. Of the 6 amino acids possible at HLA-DRB1 position 11, Val, Ser, and Leu were most prevalent in the total RA population (prevalence of 33.2%, 31.7%, and 14.0%, respectively). Within the ACPA-positive group of RA patients, Val was the most prevalent amino acid (41.6%), and within the ACPA-negative group, Ser was the most prevalent (43.7%). At HLA-B position 9, Tyr was the most prevalent and Asp the least prevalent amino acid (prevalence of 62.4% and 12.6%, respectively). At HLA-DPB1 position 9, Phe was the most common amino acid with a prevalence of 74.4%. For HLA-B and HLA-DPB1, the frequencies of amino acids were similar for ACPA-positive and ACPA-negative RA.

HLA-DRB1 SE alleles

First, the association between the HLA-DRB1 SE alleles and radiographic progression was studied. As expected, the SE alleles were associated with more severe radiographic progression ($p=6.41x10^{-7}$ in the fixed-effects meta-analysis of the 4 cohorts) (Figure 2). When SE and ACPA were analysed concomitantly, the significance of the SE alleles was lost (meta-analysis p=0.20) and ACPA was still significant (meta-analysis $p=2.22x10^{-16}$). The association between the SE alleles and radiographic progression was thus not independent of ACPA, suggesting that ACPA play a role in the causal path of the SE alleles and radiographic progression.

HLA-DRB1 position 11

Patients with the risk amino acids Val and Leu had a higher rate of joint damage progression; this reached statistical significance in 3 individual cohorts (EAC $p=4.94x10^{-5}$, Umeå p=0.032, and NDB p=0.013) and in the meta-analysis ($p=5.11x10^{-7}$) (Figure 2). To illustrate, the raw radiographic data for RA patients included in the Leiden EAC are presented in Figure 3. In these patients, the presence of one Val or Leu amino acid was associated with a 1.033-fold rate of joint destruction per year compared to patients without these amino acids; this equals a 26% (1.033 to the power 7) higher rate of joint destruction over 7 years. To further illustrate the effects, RA patients with 1 and 2 Val or Leu amino acids, respectively, had SHS scores that were 10 and 16.5 points higher than patients without any Val or Leu amino acids (Figure 3). Conditioning on SE status revealed an independent association of position 11 with radiographic progression (meta-analysis p=0.022) (Figure 2). This indicates that Val or Leu on HLA-DRB1 position 11 was associated with radiographic progression independent of the HLA-DRB1 SE status.

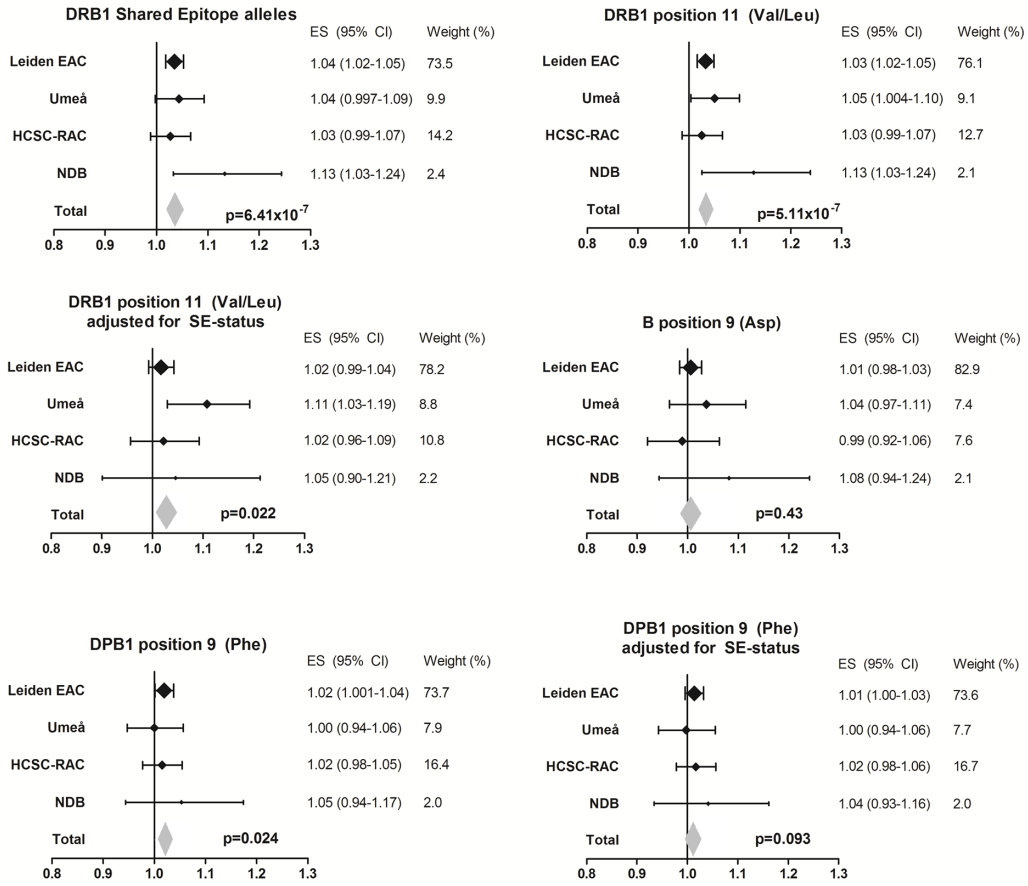

Figure 2. Associations of amino acids at HLA-DRB1, HLA-B, and HLA-DPB1 that are known to predispose to RA development with radiographic progression in RA. The yearly radiographic progression rates per risk amino acid in each individual cohort are shown. P-values are from the fixed-effects meta-analyses evaluating the 4 cohorts, which consisted of a total of 1,878 patients and 4,911 sets of radiographs. Risk amino acids were defined according to the findings of Raychaudhuri et al [10]. For the DRB1 shared epitope (SE), I^2 22.9%, p=0.27, fixed-effects p=6.41x10^{-7}, and random-effects p=2.01x10^{-4}. For Val/Leu at DRB1 position 11, I^2 23.0%, p=0.27, fixed-effects p=5.11x10^{-7}, and random-effects p=2.19x10^{-4}. For Val/Leu at DRB1 position 11 adjusted for SE status, I^2 37.3%, p=0.19, fixed-effects p=0.022, and random-effects p=0.066. For Asp at B position 9, I^2 0.0%, p=0.59, fixed-effects p=0.43, and random-effects p=0.43. For Phe at DPB1 position 9, I^2 0.0%, p=0.85, fixed-effects p=0.024, and random-effects p=0.024. For Phe at DPB1 position 9 adjusted for SE status, I^2 0.0%, p=0.90, fixed-effects p=0.093, and random-effects p=0.093. ES=effect size.

In order to determine whether the effect of Val and Leu at position 11 on radiographic progression was independent of the effect of ACPA on radiographic progression, analyses were also adjusted for ACPA status. Similar to the SE alleles, the effect of Val and Leu on radiographic progression was not independent of ACPA status (meta-analysis p=0.12).

We next studied amino acid Ser at HLA-DRB1 position 11 in more detail, since this variant was reported to protect against RA [10]. Analysis showed that Ser was associated with

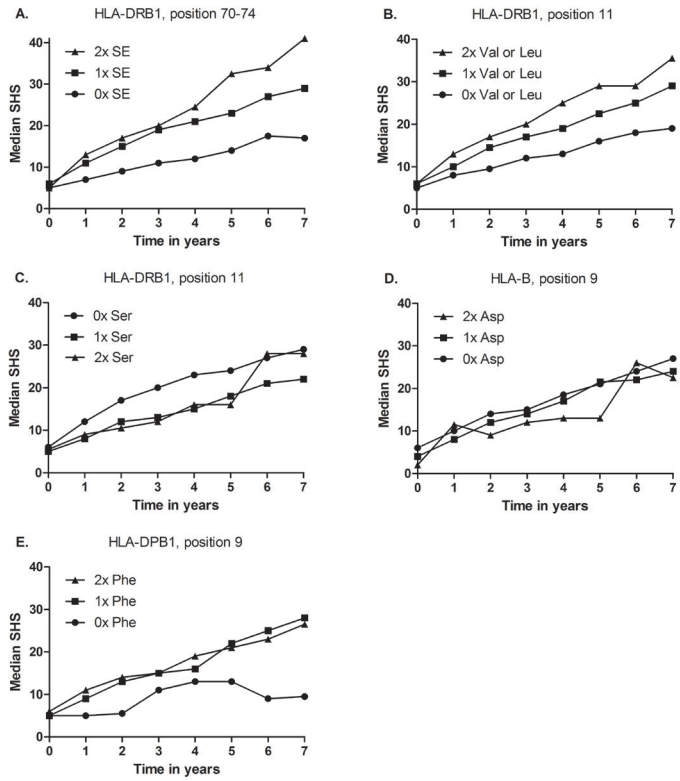

Figure 3. Radiographic progression in RA patients in the Leiden EAC cohort per number of risk alleles or amino acids at HLA-DRB1, HLA-B, and HLA-DPB1. The median raw and unmodeled Sharp-van der Heijde scores (SHS) during 7 years of follow-up of 594 RA patients are shown. The p-values obtained by multivariate normal regression analyses comparing the genotypes in relation to radiographic progression were $p=5.33x10^{-5}$ for the DRB1 shared epitope (SE) alleles, $p=4.94x10^{-5}$ for Val/Leu at DRB1 position 11, p=0.044 for Ser at DRB1 position 11, p=0.62 for Asp at B position 9, and p=0.036 for Phe at DPB1 position 9.

a lower rate of joint destruction (meta-analysis $p=5.99x10^{-3}$) (Figure 4A). Because of the polymorphic nature of HLA-DRB1 and because predisposing as well as protective associations of variants encoded by the same position with radiographic progression were observed, we subsequently performed stratified analyses to distinguish between true-positive signals (i.e., a truly predisposing variant) and false-positive signals (i.e., seemingly predisposing but actually caused by the absence of protective variants). First, the association of Val and Leu was studied when comparing patient groups with S/S, S/N and N/N, thus excluding patients with the protective amino acid Ser. Carrying Val and Leu was still associated with more radiographic progression (meta-analysis p=0.010) (Figure 5A), and this association remained after additionally conditioning on SE status (meta-analysis p=0.012) (Figure 5B), indicating a truly predisposing association for Val and Leu with radiographic progression. Furthermore, when performing stratified analysis to evaluate the effect of Ser in patients not carrying the predisposing variants Val and Leu (thus comparing 3 groups of patients: P/P, P/N, and N/N) the protective effect of Ser was lost (meta-analysis p=0.48) (Figure 4B). This might suggest

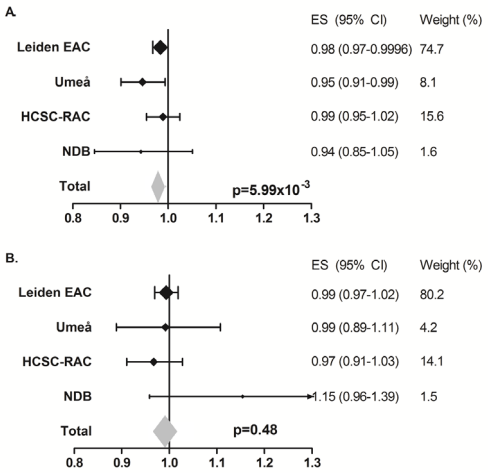

Figure 4. Association of Ser at HLA-DRB1 position 11 with radiographic progression of RA in the total population (A) and in the subgroup of patients who did not carry any predisposing Val or Leu amino acids (B). (A) Yearly radiographic progression rates per Ser amino acid at HLA-DRB1 in the total population. Data are shown for each individual cohort and for the fixed-effects meta-analyses evaluating the 4 cohorts, consisting of a total of 1,878 patients and 4,911 sets of radiographs. I^2 0.0%, p=0.40, fixed-effects p=5.99x10^{-3}, and random-effects p=5.99x10^{-3}. (B) Yearly radiographic progression rates per Ser amino acid at HLA-DRB1 in the patients who did not carry any predisposing Val or Leu amino acids. To determine whether the observed association of Ser with radiographic progression was truly protective and not due to the concomitant absence of the predisposing amino acids Val and Leu, analyses were performed within the subgroup of patients not carrying Val and Leu (thus, patients with 2 copies of Ser, patients with 1 Ser and 1 neutral amino acid, and patients with 2 neutral amino acids were compared). Data are shown for each individual cohort and for the fixed-effects meta-analyses evaluating the 4 cohorts, consisting of a total of 541 patients and 1,477 sets of radiographs. I^2 9.5%, p=0.35, fixed-effects p=0.48, and random-effects p= 0.56.

that the protective effect of Ser observed in the total RA population was the consequence of the absence of the predisposing amino acids Val and Leu rather than a truly protective effect.

HLA-B position 9

Because risk factors for RA located outside the HLA-DRB1 region were also identified, we subsequently studied the association of 2 amino acids that are located elsewhere in the HLA region. First, the association between amino acid Asp at HLA-B position 9 and radiographic progression was assessed. No association was observed, neither in the individual cohorts nor in a meta-analysis (meta-analysis p=0.43). Also, no tendency for association was observed (Figure 2) (Figure 3 illustrates the raw radiographic data for HLA-B position 9 in the Dutch RA patients).

HLA-DPB1 position 9

The other variant that is not located within the HLA-DRB1 region was HLA-DPB1. Amino acid Phe at position 9 in HLA-DPB1 was significantly associated with more severe radiographic progression (meta-analysis p=0.024) (Figure 2) (Figure 3 illustrates raw radiographic data for RA patients in the EAC). However, after conditioning on SE status, no significance was obtained (meta-analysis p=0.093) (Figure 2). Furthermore, when conditioning the effect of

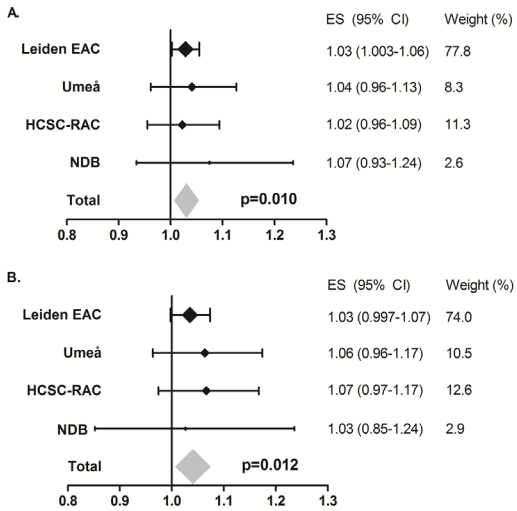

Figure 5. Association of Val and Leu at HLA-DRB1 position 11 with radiographic progression in the subgroup of RA patients who did not carry any Ser at position 11 (A) and with additional adjustment for shared epitope (SE) status (B). (A) Yearly radiographic progression rates per Val or Leu amino acid at HLA-DRB1 position 11 in patients who did not carry any Ser at position 11. These analyses were performed to determine whether the observed association of Val and Leu with radiographic progression was truly predisposing and not due to the concomitant absence of Ser (thus, patients carrying 2 copies of Val or Leu, patients with 1 Val or Leu and 1 neutral amino acid, and patients with 2 neutral amino acids were compared). Data are shown for each individual cohort and for the fixed-effects meta-analyses evaluating a total of 781 patients and 1,747 sets of radiographs. I^2 0.0%, p=0.92, fixed-effects p=0.010, and random-effects p=0.010. (B) Yearly radiographic progression rates per Val or Leu amino acid at HLA-DRB1 position 11 in patients who did not carry any Ser at position 11, adjusted for SE status. I^2 0.0%, p=0.90, fixed-effects p=0.012, and random-effects p=0.012.

Phe for ACPA significance was lost (meta-analysis p=0.27).

Predisposing variants within ACPA-negative RA

Recently, the amino acids Leu and Ser on HLA-DRB1 position 11 and Asp on B position 9 were identified as risk factors for ACPA-negative RA [11]. Associations between these amino acids and radiographic progression were also studied within the ACPA-negative subgroup, consisting of 657 patients with 1,877 radiograph sets. No significant results were obtained for Leu/Ser at HLA-DRB1 position 11 or Asp at HLA-B position 9 (meta-analysis p=0.18 and p=0.28, respectively). Also, the directionality of the effects was diverse (Figure 6) (see Supplementary Figure 2).

DISCUSSION

This study was undertaken to investigate whether genetic variants at HLA-DRB1 position 11, HLA-B position 9, and HLA-DPB1 position 9, which were recently identified as risk factors for RA, were also associated with radiographic progression in RA, and whether the associations were independent of HLA-DRB1 SE status and ACPA status. We observed

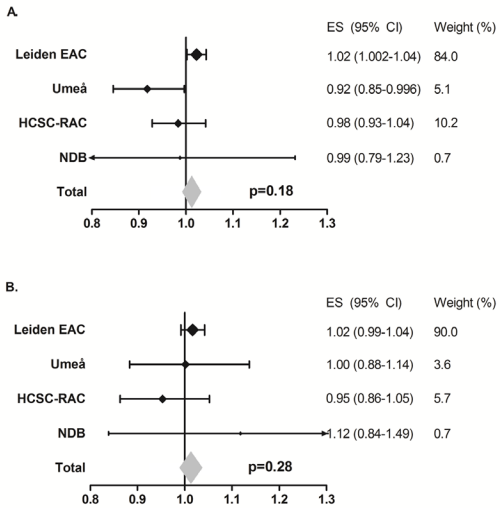

Figure 6. Association of Leu and Ser at HLA-DRB1 position 11 and Asp (A) at HLA-B position 9 (B) with radiographic progression in ACPA-negative RA. Data are shown for each individual cohort and for the fixed-effects meta-analyses evaluating the 4 cohorts, consisting of a total of 657 patients and 1,877 sets of radiographs. Risk amino acids were defined according to the findings of Han et al [11]. (A) Yearly radiographic progression rates per risk amino acid Leu or Ser at HLA–DRB1 position 11 in ACPA-negative patients. I^2 60.8%, p=0.054, fixed-effects p=0.18, and random-effects p=0.59. (B) Yearly radiographic progression rates per risk amino acid Asp at HLA-B position 9 in ACPA-negative patients. I^2 0.0%, p=0.56, fixed-effects p=0.28, random-effects p=0.28.

that RA patients carrying the amino acids Val or Leu at HLA-DRB1 position 11 had more severe radiographic progression. Despite linkage between the variants at position 11 and the HLA-DRB1 SE alleles, the effect of Val and Leu was independent of the known SE effect. In addition, similar to the SE alleles, the effect of Val and Leu at HLA-DRB1 position 11 was not independent of ACPA. Therefore, the findings of the present study validate the relevance of these amino acids at position 11 in HLA-DRB1.

Identifying which variants in the HLA region are associated with the development or course of RA provides the opportunity to increase the understanding of the mechanisms underlying the progression of RA. The recent observation that the genetic variants that are associated with risk of RA development and those that are associated with risk of a progressive course of RA are largely different suggests that the processes driving disease development and disease severity are to a large extent dissimilar [1]. However, a few genetic variants are risk factors for both RA development and RA severity, highlighting the importance of the pathway indicated by these risk factors throughout the disease. One such risk factor is the presence of the SE alleles encoded by HLA-DRB1 positions 70-74, which act via ACPA on disease development and outcome. The findings of the present study demonstrate that Val and Leu at HLA-DRB1 position 11 are also associated with both the development and severity of RA as measured by radiographic progression.

The hope of the formulation of the SE hypothesis was that it would allow the

identification of the autoantigens of RA [6]. However, thus far, arthritogenic peptides have not been found. Mouse studies have revealed that small changes in amino acids located within the antigen-presenting binding site may have large influences on the antigens that are presented [12,13]. The amino acids at position 11 and position 13 are observed to be in very tight linkage disequilibrium [10]; therefore, only the amino acids of position 11 were analysed. Future studies will reveal whether taking the amino acids at position 11 and 13 into account will be helpful in identifying the pathogenic antigens that result in immune activation and autoantibody production, thereby stimulating disease development or progression.

Investigation of the HLA region is challenging due to high linkage disequilibrium. This is illustrated by the fact that 91.8% of all patients evaluated in the present study that had 0, 1, or 2 SE alleles also had, respectively, 0, 1, or 2 Val/Leu amino acids at HLA-DRB1 position 11, implying that the observation of an additive value of position 11 was actually based on a relatively small proportion of patients. In addition, the association between SE and radiographic progression is strong, providing another reason why large datasets are required to determine whether other HLA variants have additional effects that are independent of the SE effect. Thus far, to the best of our knowledge, no studies have been published providing replication of the results regarding RA susceptibility published by Raychaudhuri et al in completely independent datasets [10]. Also in this respect, the findings of the present study, though evaluating a different outcome, support the relevance of position 11 on HLA-DRB1.

In addition to linkage and power issues, the polymorphic nature of the HLA-DRB1 region in which the same position can encode for both predisposing and protective variants adds another level of complexity. To unravel these effects, adjusted multivariable analyses and stratified analyses can be done. The advantage of adjusting in multivariable analyses is that it is more powerful than subgroup analyses; however, this is at the cost of possible disturbing effects due to the assumptions underlying the model and eventual disturbances due to multicollinearity. In our view, in the present setting, stratified analyses yield the most conclusive results. We observed that Val and Leu at HLA-DRB1 position 11 were truly predisposing to more severe disease. In contrast, our data suggested that carrying the amino acid Ser at the same position was not protective. Although a protective effect was observed initially, in stratified analyses (excluding the effect of the absence of predisposing effects of Val and Leu), Ser was not significantly associated with less severe radiographic progression. These data might suggest that Ser does not have a protective effect on the severity of radiographic progression in RA. However, it is also possible that the present study was insufficiently powered to detect a protective effect of Ser in the subgroup of patients studied. We cannot discriminate between these two possible explanations, and further studies are needed to determine the association of Ser with the severity of radiographic progression.

Raychaudhuri et al also defined 16 haplotypes, based on positions 11, 71, and 74 in HLA-DRB1 [10]. We did not perform haplotype analyses, since in our view stratification is needed to distinguish predisposing or protective haplotypes from the absence of protective

and predisposing haplotypes, respectively, and then subgroups became too small.

Within the field of RA susceptibility, it has been notable that Ser has been reported to be protective against ACPA-positive RA but predisposing to ACPA-negative RA [10,11]. Because of this finding, we also evaluated the association of Ser with progression in the ACPA-negative subgroup. The presence of Ser in these patients was not associated with the severity of radiographic progression.

We could not identify an association between Asp at HLA-B9 and radiographic progression. This position, which is highly correlated with HLA-B*08, is part of the conserved ancestral A1-B8-DRB1*03 (8.1) haplotype and has been associated with susceptibility to ACPA-negative disease [8,9]. In contrast to the undisputed association of DRB1*03 or this haplotype with susceptibility to ACPA-negative RA, we did not detect an association with the severity of the disease in RA or in ACPA-negative RA.

The presence of Phe at HLA-DPB1 position 9 was associated with the severity of radiographic progression, but significance was lost (p=0.093) when conditioning for the HLA-DRB1 SE status. This might suggest that Phe at HLA-DPB1 is not associated with radiographic progression independent of the association between SE status and radiographic progression. Notably, the directionality of the effect of Phe at HLA-DPB1 was similar in all cohorts, with more severe progression in the patients carrying Phe. Although the linkage between this position with the HLA-DRB1 SE alleles is less than for HLA-DRB1 position 11 with SE (in our study 32.7% of the patients who had 0, 1, or 2 SE alleles also had, respectively, 0, 1, or 2 Phe amino acids at HLA-DPB1 position 9), it is possible that our data were insufficiently powered to find an independent association of Phe with radiographic progression. The present data do not allow making definite conclusions regarding HLA-DPB1 and radiographic progression; though if an effect is present, this effect seems to act in the path of ACPA, similar to HLA-DRB1.

In order to get some indication of the variance in joint destruction explained by the genetic factors studied, the interindividual variance in joint destruction in the Leiden RA patients at year 7 was evaluated. The SE alleles explained 4.2% of the variance in joint destruction and the combination of SE alleles, HLA-DRB1 position 11, HLA-DPB1 position 9, and HLA-B position 9 explained 4.5%. This is lower than the variance in the risk of ACPA-positive RA explained by a combination of HLA-DRB1 positions 71, 74, and 11, HLA-DPB1 position 9, and HLA-B position 9, which was reported to be 12.7%[10].

To conclude, Val and Leu at position 11 in the HLA-DRB1 locus were recently identified as additional susceptibility factors for ACPA-positive RA. We observed that these amino acids were also associated with a more severe disease course, an effect that was not independent of ACPA status. Further 3-dimensional modeling studies or binding assays, such as described previously by Scally et al [27] are needed to determine whether the present findings result in novel insights in the consequences of the HLA-DRB1 motif on antigen presentation

and its function as an immune response gene.

SUPPLEMENTARY DATA

Supplementary data are published on the web site of *Arthritis & Rheumatology.*

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IL2RA is associated with persistence of rheumatoid arthritis

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ABSTRACT

Introduction

Although rheumatoid arthritis (RA) is generally a chronic disease, a proportion of RA patients achieve disease-modifying antirheumatic drug (DMARD)-free sustained remission, reflecting loss of disease-persistence. To explore mechanisms underlying RA persistence, we performed a candidate gene study. We hypothesised that variants associating with lack of radiographic progression also associate with DMARD-free sustained remission.

Methods

645 Dutch RA patients were studied on DMARD-free sustained remission during a maximal follow-up duration of 10 years. Variants associated with radiographic progression under an additive model in the total RA population (*Human Leukocyte Antigens (HLA)-DRB1*-shared epitope (SE), *Dickkopf-1 (DKK1)*-rs1896368, *DKK1*-rs1896367, *DKK1*-rs1528873, *C5Orf30*-rs26232, *Interleukin-2 receptor-α* (*IL2RA*)-rs2104286, *Matrix metalloproteinase-9* (*MMP-9*)-rs11908352, rs451066 and *Osteoprotegerin* (*OPG*)-rs1485305) were studied. Cox-regression analyses were performed and Bonferroni correction applied. Soluble IL2Rα (sIL2Rα)levels were studied. For replication, 622 RA patients included in the French Evaluation et Suivi de POlyarthrites Indifférenciées Récentes cohort (ESPOIR) cohort were investigated. Results were combined in inverse-variance weighted meta-analysis.

Results

Similar as previously reported, the SE-alleles associated with less remission (hazard ratio (HR)=0.57, 95% confidence interval (95% CI)= 0.42-0.77, $p=2.72x10^{-4}$). Variants in *DKK-1*, *C5Orf30*, *MMP-9* and *OPG* were not associated with remission. The *IL2RA* rs2104286 minor allele associated with a higher chance on remission (HR=1.52, 95% CI=1.16-1.99, $p=2.44x10^{-3}$). The rs2104286 minor allele associated with lower sIL2Rα levels ($p=1.44x10^{-3}$); lower sIL2Rα levels associated with a higher chance on remission (HR per 100 pg/L=0.81, 95% CI=0.68-0.95, p=0.012). When including rs2104286 and sIL2Rα levels in one analysis, the HR for rs2104286 was 2.27 (95% CI=1.06-4.84, p=0.034) and for sIL2Rα 0.83 (95% CI=0.70-0.98, p=0.026). Within ESPOIR, the HR of rs2104286 was 1.31 (95% CI=0.90-1.90). The meta-analysis revealed a p-value of 1.01×10^{-3}.

Conclusion

IL2RA rs2104286 and sIL2Rα level associated with RA persistence. *IL2RA* variants are known to protect against multiple sclerosis, diabetes mellitus and RA. Besides *HLA*-SE, *IL2RA* rs2104286 is thus far the only known genetic variant associated with both joint destruction and RA persistence. This underlines the relevance of *IL2RA* for RA.

Introduction

Persistent inflammation and progression of joint damage are the two hallmarks of rheumatoid arthritis (RA). At present, clinically relevant joint destruction has become infrequent owing to modern treatment strategies. Despite this improvement, RA is still a chronic disease in the majority of patients. Some patients, however, are able to stop taking disease-modifying antirheumatic drugs (DMARDs) without restart of DMARD treatment and without recurrence of arthritis; this is called DMARD-free sustained remission. This disease remission reflects the opposite of RA persistence and frequencies of DMARD-free sustained remission are reported to vary between 5 and 22 % [1–5]. A thorough comprehension of the mechanisms promoting disease persistence is required to derive targeted interventions aiming to reduce the chronic nature of RA. At present, however, the biologic mechanisms underlying disease persistence are largely unknown.

Only a few risk factors for RA persistence (absence of achieving DMARD-free sustained remission) have been reported and replicated. One of these factors is prolonged symptom duration at treatment start [1,4,6,7]. This risk factor points to a so-called "window of opportunity" in RA but the processes underlying this association are unidentified. Another risk factor is the presence of RA-related autoantibodies [1,2]. Although it is not exactly known how these autoantibodies exert their effect, several possibilities have been proposed [8]. However, the presence of rheumatoid factor (RF) or anti-citrullinated peptide antibodies (ACPA) explain only a proportion of the variance in achieving DMARD-free remission as the large majority of autoantibody negative RA patients have persistent disease and some patients with autoantibodies can achieve remission [9]. One genetic risk factor has been found associated with arthritis persistence in two European cohorts: the presence of *human leukocyte antigen* (*HLA*)-*DRB1* shared epitope (SE) alleles. This risk factor presumably acts in the same pathway as ACPA [1,2].

To increase the understanding of processes underlying disease persistence, it is valuable to study patients who have achieved DMARD-free sustained remission over time, because this reflects loss of disease persistence. This study aimed to identify further risk factors for achieving DMARD-free sustained remission. To this end, a candidate gene study was performed. To select genetic candidates, we hypothesised that genetic variants which are associated with a lack of radiographic joint damage also associate with DMARD-free sustained remission. Nine variants reported to associate with radiographic progression using an additive model in the total RA population were studied in relation to DMARD-free sustained remission in an observational cohort of 645 Dutch RA patients with a maximal follow-up of 10 years. Significant associations were evaluated for replication in a second cohort, comprising 622 French RA patients. One of the nine studied variants was the already known risk factor *HLA-DRB1* SE [1]; this variant was included in the present study for a complete overview. Another interesting gene is *interleukin-2 receptor alpha* (*IL2RA*);

variants in *IL2RA* have shown to be associated with a decreased risk for development of RA [10,11] and for the development of other autoimmune diseases such as multiple sclerosis (MS) [12] and diabetes mellitus (DM) [13,14]. Furthermore, rs2104286 in *IL2RA* is, apart from the *HLA* SE, the only genetic factor that associates with the risk of RA development [10] and with the severity of radiographic progression within RA [15].

METHODS

Patients

RA patients fulfilling the 1987 American College of Rheumatology (ACR) criteria for RA and included in two European cohorts were studied. All patients gave their informed consent, and approval was obtained from the local medical ethics committees.

Leiden Early Arthritis Clinic cohort - A total of 645 RA patients who were included between 1993 and 2008 were studied. The Leiden Early Arthritis Clinic (EAC) is a Dutch population-based inception cohort that started in 1993 and has been described previously [2]. Consecutively referred patients were included when arthritis was present at physical examination and symptom duration was <2 years. The initial treatment strategy was different for patients included and diagnosed during different inclusion periods: patients included in 1993-1995 were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and then DMARDs were initiated with delay; patients included in 1996-1998 were treated early with rather mild DMARDs such as hydroxychloroquine or sulfasalazine; and patients included in 1999-2008 were treated promptly with methotrexate [2].

Evaluation et Suivi de POlyarthrites Indifférenciées Récentes cohort - Evaluation et Suivi de POlyarthrites Indifférenciées Récentes (ESPOIR) is a prospective cohort study that started in 2002, including patients with RA or a suspicion to develop RA from 14 French rheumatology centers. Patient can be included if aged 18-70 years and at least two swollen joints are present for >6 weeks and <6 months [16]. In total, 622 RA patients consecutively included between 2002 and 2005 were studied.

In both cohorts at baseline and at the yearly follow-up visits, questionnaires were completed, physical examination was performed, and serum samples and radio-graphs were taken [2,16.]

Outcome

DMARD-free sustained remission was defined as the sustained absence of arthritis (by physical examination) after discontinuation of DMARD therapy, including biologics and glucocorticoids (systemic and intra-articular). In the Leiden EAC cohort, arthritis had to be absent for the entire follow-up period and at least during 1 year. For patients with a follow-up longer than 10 years, the follow-up duration studied was restricted to 10 years. Medical files of all patients were studied on remission, and this was determined until 5 April 2012.

Patients who achieved DMARD-free sustained remission initially but relapsed later over time (n=2) did not fulfill the criterion that arthritis should remain absent during the total follow-up period and were included in the non-remission group. In the ESPOIR cohort, the follow-up was shorter and restricted to 5 years. To be classified as having DMARD-free sustained remission, arthritis had to be absent during at least 1 year after cessation of DMARDs but not necessarily during the rest of the follow-up. Here the outcome was assessed reviewing the structured visits in the database; medical files were not explored.

Single nucleotide polymorphism selection and genotyping

Single Nucleotide Polymorphisms (SNPs) selection for the present study was based on a recently performed literature review on genetic variants in relation to radiographic progression [17] and the following criteria were used: (1) the SNP has been reported and replicated or found significant in meta-analysis of several cohorts to associate with radiographic joint damage progression. Furthermore, the observed association with radiographic progression was done (2) using an additive model and (3) in the total RA population and not confined to either the ACPA-positive or ACPA-negative subgroup. The latter two criteria were included because it was expected that performing analyses on DMARD-free sustained remission using a recessive model (in which the group of patients with two minor alleles is in general small) or in only a subgroup of patients with or without autoantibodies would have insufficient power to reach statistical significance. This expectation was substantiated by power analyses (calculated using PASS 11; NCSS, Kaysville, UT, USA) based on our cohort (645 RA patients and 332 ACPA-positive patients). These analyses revealed that for an 80 % power study for an additive association in the total RA population, a hazard ratio (HR) of 1.5 would be required, but for an 80 % power study for a recessive association in the total RA population or for an additive association in only the ACPA-positive subgroup, HRs would be required of respectively 2.5 and 3.1 which are too high to be expected of SNP effects because SNPs generally have low effect sizes [11].

Based on the criteria, nine genetic variants were selected for evaluation in the Leiden EAC cohort: SE in *HLA-DRB1* [18], rs1896368, rs1896367, and rs1528873 in *Dickkopf-1* (*DKK-1*) [19], rs2104286 in *IL2RA* [15], rs26232 in *C5Orf30* [20], rs11908352 in *matrix metalloproteinase-9* (*MMP-9*) [21], rs451066 at chromosome 14 [21], and rs1485305 in *osteoprotegerin* (*OPG*) [22]. Newly identified SNPs that were significantly associated with DMARD-free sustained remission in the Leiden EAC cohort were selected for evaluation in the ESPOIR cohort.

Within the Leiden EAC cohort, the *HLA-DRB1* alleles were genotyped using two-digit typing which was complemented by four-digit typing of the *DRB1*04* alleles and by specific probes to detect the presence of the SE sequences in individuals carrying *DRB1*01* or *DRB1*10* alleles 1. The following alleles were classified as SE alleles: *DRB1*0101*, *DRB1*0102*, *DRB1*0104*, *DRB1*0401*, *DRB1*0404*, *DRB1*0405*, *DRB1*0408*, *DRB1*0413*, *DRB1*0416*, *DRB1*1001*, and *DRB1*1402* [23]. Genotyping data on rs1896368, rs1896367, and rs1528873 in

DKK-1 and on rs1485305 in *OPG* were retrieved using Illumina's Golden Gate platform with an overall error rate <2.5 % and success rates >95% [19,22]. rs26232 in *C5Orf30* was genotyped using LightSnp (Roche) with an overall error rate <1% and success rates >99% [20]. Genotyping data on rs2104286 in *IL2RA*, rs11908352 in *MMP-9*, and rs451066 at chromosome 14 were retrieved using Illumina's Immunochip with an overall error rate <1 % and success rates >98%. Hardy-Weinberg equilibrium for all SNPs was p >0.001 [15,21].

Within the ESPOIR cohort, rs2104286 was genotyped used allele-specific kinetic polymerase chain reaction ana-lysis by KBiosciences (UK) using the KASPar method. The success rate was 97.9 % as described previously [24].

Soluble IL2Rα

In 159 Dutch RA patients, soluble interleukin-2 receptor alpha (sIL2Rα) levels were evaluated using the standard sandwich enzyme-linked immunosorbent assay (ELISA) for sIL2Rα. The ELISA was performed according to the manufacturer's recommendations (BD Biosciences). The serum levels were determined for a previous study on *IL2RA* [15]. Samples were collected at a median disease duration of 4 years (range 1-9 years). For patients who achieved remission, the sIL2Rα level was determined in samples taken before remission was achieved.

Statistical analysis

Cox proportional hazard regression analyses were carried out with DMARD-free sustained remission as the outcome. The date of remission was defined as 1 year after the date at which DMARDs were withdrawn owing to remission of disease. Time to remission was the time from date of inclusion to the date of remission. Patients who did not achieve remission were censored at the date when all medical files were studied on the achievement of DMARD-free sustained remission (5 April 2012). Analyses were adjusted for age, gender, and inclusion period (a proxy for the differences over time in applied treatment strategies), similar to previous reports [2,3,19]. Genotypes were tested additively. The association of sIL2Rα levels (continuous variable) was tested similarly with an additional adjustment for disease duration at the time of sample collection. For the genetic variants, the cut-off for statistical significance was set at $p<5.56x10^{-3}$ (0.05/9 tests) using the Bonferroni correction for multiple testing. For the test on the serum level, $p<0.05$ was considered significant. In the ESPOIR cohort, a similar Cox proportional hazard regression analysis adjusted for age and gender was performed. ESPOIR RA patients were diagnosed in a relatively short interval and no adjustments were made for initial treatment strategies. Results of the two cohorts were combined in an inverse-variance weighted meta-analysis. Analyses were performed using IBM SPSS version 20 (Armonk, NY, USA) and STATA version 12 (College Station, TX, USA).

RESULTS

Patients

The baseline characteristics of the 645 studied RA patients in the Leiden EAC cohort are

presented in Table 1. During the median follow-up duration of 8.6 years (interquartile range (IQR) 5.5-10.0 years), 119 patients achieved DMARD-free sustained remission. The incidence rate for achieving remission was 2.4 per 100 person-years (119 events during the total follow-up of all patients of 4,885 years). The patients who achieved remission did so after a median disease duration of 4.3 years (IQR 2.9-6.1 years). Patients who achieved DMARD-free sustained remission had shorter symptom duration at disease onset (median 12.9 versus 20.3 weeks, p<0.001) and had less frequent autoantibodies (ACPA-positivity, 13.0 % versus 61.3 %, p<0.001; RF-positivity, 27.1 % versus 64.7 %, p<0.001) compared with patients who did not achieve remission (Table 1).

Genetic variants and achieving DMARD-free sustained remission

Presence of the SE alleles was significantly associated with DMARD-free sustained remission ($p=2.72\times10^{-4}$). The HR per SE allele on achieving DMARD-free sustained remission was 0.57 (95% confidence interval (95% CI)=0.42-0.77) compared with patients without SE alleles (Table 2; Additional file 1); this finding is in line with previous reports.

Rs1896368, rs1896367, and rs1528873 (all *DKK-1*), rs26232 (*C5Orf30*), rs11908352 (*MMP-9*), rs451066 (chromosome 14), and rs1485305 (*OPG*) were not associated with

Table 1. Patient characteristics of the Leiden EAC

	Total (n=645)	DMARD-free sustained remission achieved during follow-up (n=119)	DMARD-free sustained remission not achieved during follow-up (n=526)
Baseline			
Age (years), mean (SD)	56.9 (15.6)	58.8 (16.9)	56.5 (15.3)
Female, n (%)	430 (66.7)	74 (62.2)	356 (67.7)
Symptom duration (weeks), median (IQR)	18.8 (10.3-37.3)	12.9 (7.3-28.6)	20.3 (11.4-40.0)
66 Swollen joint count, median (IQR)	8 (4-13)	9 (4-15)	8 (4-13)
CRP level in mg/L, median (IQR)	18 (8-42)	18 (8-43)	18 (7-37)
ACPA-positive, n (%)	332 (52.5)	15 (13.0)	317 (61.3)
RF-positive, n (%)	371 (57.8)	32 (27.1)	339 (64.7)
Follow-up			
Duration until DMARD-free sustained remission in years, median (IQR)	4.3 (2.9-6.1)	N/A	

Data was missing on swollen joint count in 7 patients, on CRP level in 29 patients, on ACPA in 13 patients, on RF in 3 patients and on symptom duration in 47 patients.
The median symptom duration and the frequencies of ACPA-positivity and RF-positivity were significantly different between patients that achieved and not achieved DMARD-free sustained remission during follow-up (all p<0.001). The other baseline characteristics did not differ between the groups.

DMARD-free sustained remission (Table 2). Rs2104286 in *IL2RA* significantly associated with achieving DMARD-free sustained remission ($p=2.44x10^{-3}$). The HR per minor C allele for achieving DMARD-free sustained remission was 1.52 (95% CI=1.16-1.99) compared with the reference genotype of patients who were homozygous for the major T allele (Table 2 and Fig. 1); hence patients with the minor allele had an increased chance of achieving remission and, as reported earlier [15], less radiographic progression.

Table 2. Genetic risk factors for severity of joint damage in relation to achieving DMARD-free sustained remission in the Leiden EAC

Genetic variant (minor allele)	Located in/nearby gene(s) (chrom)	MAF	HR per minor allele (95% CI)	p-value
Shared Epitope [18]	*HLA-DRB1* (6)	39.6%	0.57 (0.42-0.77)	$2.72x10^{-4}$
rs1896368 (G) [19]	*DKK-1* (10)	45.8%	0.98 (0.75-1.28)	0.88
rs1896367 (A) [19]		41.5%	0.96 (0.73-1.25)	0.75
rs1528873 (C) [19]		46.7%	1.21 (0.93-1.58)	0.15
rs2104286 (C) [15]	*IL2RA* (10)	24.3%	1.52 (1.16-1.99)	$2.44x10^{-3}$
rs26232 (T) [20]	*C5orf30* (5)	28.9%	1.08 (0.81-1.44)	0.61
rs11908352 (A) [21]	*MMP-9* (20)	20.9%	0.78 (0.56-1.09)	0.15
rs451066 (A) [21]	rs1465788 (14)	19.6%	0.87 (0.63-1.21)	0.41
rs1485305 (T) [22]	*OPG* (8)	44.2%	1.00 (0.76-1.32)	0.98

Analyses were adjusted for age, gender and inclusion period (as proxy for treatment strategy).

Genetic variants and achieving DMARD-free sustained remission in relation to ACPA status

Because genetic risk factors for ACPA-positive and ACPA-negative RA are different and ACPA-positive and ACPA-negative RA are considered separate disease entities, we studied whether the observed associations were independent of ACPA or were restricted to a subset of RA patients. The analyses of *HLA-DRB1* SE and rs2104286 (*IL2RA*) were therefore repeated with additional adjustment for ACPA and when stratifying for ACPA status.

When including both SE and ACPA in one analysis, SE was not significantly associated (HR=0.92, 95% CI=0.67-1.26, p=0.61) whilst ACPA remained significant (HR=0.13, 95% CI=0.072-0.22, $p=7.68x10^{-13}$), suggesting that ACPA act in the path of the SE alleles and DMARD-free sustained remission. Similarly, the SE alleles were not associated with remission in the ACPA-positive and ACPA-negative subgroups separately (p=0.84 and p=0.51 respectively; Figure S2A in Additional file 2).

Adding ACPA as additional adjustment factor in the analysis of rs2104286 (*IL2RA*) in relation to DMARD-free sustained remission revealed an HR for rs2104286 of 1.47 (95% CI=1.12-1.93, $p=5.78x10^{-3}$), suggesting that the association of rs2104286 with remission is independent of ACPA. Stratified analysis on rs2104286 in ACPA-positive and ACPA-negative subgroups showed an HR of 1.82 (95% CI=0.88-3.77, p=0.11) within ACPA-positive RA and an HR of 1.41 (95% CI=1.05-1.89, p=0.024) within ACPA-negative RA (Figure S2B in

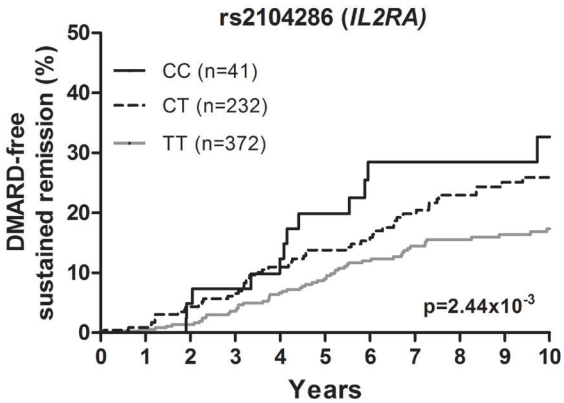

Figure 1. Rs2104286 in IL2RA in relation to achieving DMARD-free sustained remission in RA patients of the Leiden EAC cohort. Rs2104286 in IL2RA was significantly associated with achieving DMARD-free sustained remission in 645 RA patients ($p=2.44x10^{-3}$). The HR per minor C allele for achieving remission was 1.52 (95% CI=1.16-1.99). The analysis was adjusted for age, gender, and inclusion period (as a proxy for treatment strategy).

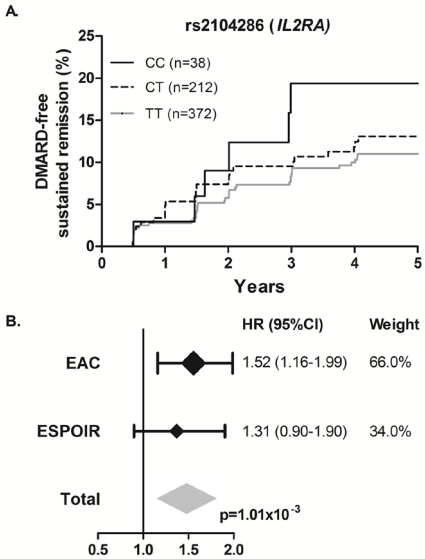

Figure 2. Rs2104286 in IL2RA in relation to achieving DMARD-free sustained remission in RA patients of the ESPOIR cohort and in meta-analysis of the Leiden EAC and ESPOIR cohorts. (A) In 622 RA patients of the ESPOIR cohort, the HR per minor C allele for achieving remission was 1.31 (95% CI=0.90-1.90, p=0.16). The analysis was adjusted for age and gender. The minor allele frequency in the ESPOIR cohort was 23.2%. (B) Results of the Leiden EAC and ESPOIR cohorts were combined in an inverse-variance weighted meta-analysis: $I^2=0.0\%$, p=0.53, fixed-effect $p=1.01x10^{-3}$, random-effects $p=1.01x10^{-3}$.

Additional file 2).

sIL2Rα levels and achieving DMARD-free sustained remission

Previous studies have shown correlations between rs2104286 in *IL2RA* and IL2Rα serum levels [25,26]. Similarly, we have previously studied rs2104286 in *IL2RA* and sIL2Rα levels in

159 RA patients from the Leiden EAC cohort and observed a significant association; the rs2104286 minor allele associated with lower sIL2Rα levels ($p=1.44x10^{-3}$) [15]. We then explored whether sIL2Rα levels were also associated with DMARD-free sustained remission and observed that lower serum levels were indeed associated with more remission (p=0.012); per 100 pg/ml increase in level, the HR of achieving remission was 0.81 (95% CI=0.68-0.95). In the 159 patients with information on sIL2Rα, rs2104286 was also associated with DMARD-free sustained remission (HR=2.57, 95% CI=1.20-5.50, p=0.015). An analysis including both rs2104286 and sIL2Rα revealed an HR of 2.27 for rs2104286 (95% CI=1.06-4.84, p=0.034) and a HR (per 100 pg/ml) of 0.83 for sIL2Rα (95% CI=0.70-0.98, p=0.026).

Replication of rs2104286 in relation to DMARD-free sustained remission in the ESPOIR cohort

Subsequently, rs2104286 in *IL2RA* was studied for replication in 622 French RA patients. The mean (standard deviation) age was 48.8 (12.3) years, 76% were female, the median (IQR) symptom duration was 22 (13-33) weeks, and 46% were ACPA-positive. After a median (IQR) follow-up duration of 5.0 (3.0-5.0) years, 67 patients achieved DMARD-free sustained remission after a median follow-up duration of 1.5 (0.7-3.0) years. The incidence rate for remission was 2.7 per 100 person-years (67 events/2451 years of total follow-up in all patients). The number of events (n=67) was lower than that of the first cohort, so the power to find significance was expected to be less than that of the first phase. Evidence of a tendency in the same direction was still considered relevant and Cox regression analyses on rs2104286 and remission were performed. The HR per minor C allele for achieving DMARD-free sustained remission was 1.31 (95% CI=0.90-1.90, p=0.16) compared with the common genotype. Although not reaching statistical significance, this indicates that, similar to the Leiden EAC cohort, patients with the minor allele had an increased chance of achieving remission (Fig. 2A). When additionally adjusting the analysis for ACPA, the HR was 1.37 (95% CI=0.95-1.97, p=0.097). Meta-analysis of the results of the Leiden EAC and ESPOIR cohorts revealed a fixed-effect p value of $1.01x10^{-3}$ (Fig. 2b).

DISCUSSION

The biological mechanisms driving disease chronicity in RA are largely unidentified. We therefore aimed to determine genetic risk factors for disease persistence in RA. Because of the low frequency of DMARD-free sustained remission (reflecting loss of disease persistence) and because of the lack of multiple large cohorts with data on this disease outcome, we were not able to perform a hypothesis-free genome-wide association study or to analyze the whole Immunochip. We used a candidate gene approach instead and hypothesised that genetic variants which associated with the severity of joint damage also associated with disease persistence. In addition to the previously reported association between the *HLA-DRB1* SE alleles and DMARD-free sustained remission (reflecting loss of disease persistence), we demonstrated that rs2104286 in *IL2RA* associated with DMARD-free sustained remission;

this minor allele that was previously associated with less severe radiographic progression [15] was associated with a higher chance of DMARD-free sustained remission. Also, the lower level of sIL2Rα observed in the presence of the rs2104286 minor allele associated with a higher chance of DMARD-fee sustained remission. Altogether the present data from two observational cohorts indicate that the *IL2RA* minor allele is not only protective for the severity of radiographic progression but also predisposes to a less persistent course of RA.

IL2RA encodes the α-chain of the high-affinity IL-2 receptor (CD25) which is expressed on and upregulated after stimulation in many immune cells, including regulatory T-cells (Tregs) [27,28]. Variants in *IL2RA* are also associated with the risk of development of RA [10,11] and other autoimmune diseases such as MS [12] and type 1 DM [13,14]. sIL2Rα is produced by proteolytic cleavage of cell-bound IL2Rα and is considered reflective of the extent of activation and expansion of T-cells [26,29,30]. Other studies reported that the minor allele of rs2104286 correlated with lower sIL2Rα levels in patients and healthy individuals [15,25,26]. In our previous study, rs2104286 was no longer associated with joint destruction after including sIL2Rα in the analysis, suggesting that the SNP might act in the same path that influenced the serum levels [15]. In present study, the genetic and serological marker remained significantly associated with DMARD-free sustained remission. This might suggest that *IL2RA* exerts part of its effect by a path which does not influence sIL2Rα levels. However, association studies cannot answer causality questions.

RA is considered to consist of ACPA-positive and ACPA-negative subentities, each with different genetic risk variants [31,32]. To determine whether the observed association was present in one or both subsets, stratified analyses were performed. Although these analyses were assumed to have insufficient power (owing to lower number of patients, and in ACPA-positive RA also a low frequency of remission), they were performed to gain insight into the data. Adjusting for ACPA is more powerful. The association of rs2104286 with DMARD-free sustained remission was independent of ACPA.

In the present study we did not fine-map the *IL2RA* region in relation to DMARD-free sustained remission because we expected to have insufficient power to find statistical significance after correcting for >400 tests. Previously the *IL2RA* region was fine-mapped in relation to joint damage progression, which is a more powerful analysis than the present survival analysis because it makes use of repeated measurements over time. rrs12722508 was identified as the SNP with the strongest association [15]. Evaluating rs12722508 in relation to DMARD-free sustained remission revealed a lower p-value and larger HR for rs12722508 (HR=1.93, $p=7.90 \times 10^{-4}$) compared with rs2104286 (HR=1.52, $p=2.44 \times 10^{-3}$). Although fine-mapping was not performed in this study, these data strengthen the finding on *IL2RA* and DMARD-free sustained remission.

At present, there is not much literature on the description of RA persistence or chronicity. In the present study, patients who were unable to reach DMARD-free sustained

remission were considered to have persistent disease. Although other definitions for RA persistence can be used, we have chosen the absence of DMARD-free sustained remission as the outcome because it is a strict definition and the closest available proxy for cure of the disease.

The majority of the studied patients had persistent disease and did not achieve remission. Recently, we reported that the chance of achieving DMARD-free sustained remission in clinical practice has become a more feasible outcome with up-to-date treatment strategies [5]. The Leiden EAC patients who were evaluated in the present study were included during the period 1993-2006. Treatment strategies have changed over time in these patients and indeed patients included in later periods had a higher chance of achieving DMARD-free sustained remission (data not shown). All analyses in the present study were adjusted for the inclusion period as a proxy for the initially applied treatment strategy, and the results obtained for *IL2RA* were thus independent of the effect of changes in treatment strategies.

Another potential limitation is that we evaluated data from longitudinal observational cohort studies. These data reflect the daily care of patients and not only decisions to start DMARDs but also decisions to stop DMARDs were left to the patients' and rheumatologists' decisions and not protocolised. In the ESPOIR cohort, mainly in the first years of its existence, quitting DMARD-therapy was uncommon. Consequently, the observed frequency of DMARD-free sustained remission may be underestimated. This may be one of the explanations contributing to a lower incidence of DMARD-free sustained remission in the ESPOIR cohort. In addition, whether DMARD-free sustained remission was achieved was determined slightly differently in the cohorts. In the Leiden EAC cohort, all medical files were checked to en-sure that DMARD-free sustained remission was present. In the ESPOIR cohort, data from the structured visits with yearly intervals were studied. It is possible that more patients included in the ESPOIR cohort would have achieved DMARD-free sustained remission when all information present in medical files was evaluated. Thirdly, the follow-up duration was shorter in the ESPOIR cohort. Differences in common practice for discontinuing DMARD-therapy, however, might be the most important cause for the higher frequency of DMARD-free sustained remission in the Leiden EAC cohort than in the ESPOIR cohort. Nonetheless, there was a strong tendency in the data from the ESPOIR cohort validating the importance of *IL2RA* for the disease course in RA.

The SE alleles were strongly associated with sustained DMARD-free remission. A similar result was previously reported (although using a dominant model instead of an additive model). We here observed that this association was not independent of ACPA, suggesting that the SE alleles act in the same path as ACPA. This finding is similar to that observed for SE, ACPA, and radiographic progression [33].

The studied variants in *DKK-1*, *C5Orf30*, *MMP-9*, and *OPG* were not associated with DMARD-free sustained remission. Although power issues might have contributed to

some negative findings, the absence of an association of these risk factors for radiographic progression with DMARD-free sustained remission suggests that the mechanisms driving joint damage progression and disease persistence are partially different.

At present >100 genetic susceptibility factors are known and several genetic risk factors for radiographic progression have been identified [11,34]. These factors were largely dissimilar; only the *HLA-DRB1* SE alleles and *IL2RA* were present in both lists of risk factors. Interestingly, the current study determined that both factors are also associated with persistence of RA. This suggests that both variants are of crucial importance for the processes mediating RA development and progression.

IL-2/IL-2 receptor signaling is important during immune responses of both effector T-cells and Tregs. Quantitatively, Tregs require less IL-2/IL-2 receptor signaling than effector T-cells to support their development and function [35,36]. Recently, the first results on immunomodulation with low-dose IL-2 in other autoimmune diseases have been published, showing efficacy on upregulation of Tregs [35,36] and improved clinical outcome [37]. Monoclonal anti-CD25 antibodies (daclizumab) have also been shown effective in reducing disease activity in autoimmune diseases [38]; this effect is not only ascribed to direct effects on T-cells but also on natural killer (NK) cells and dendritic cells [38]. To the best of our knowledge there are no data on IL-2 treatment for RA. However, if low-dose IL-2 treatment is effective, the results of IL-2 therapy in RA might also be dependent on the IL-2 receptor status of the patient, which is genetically determined. Hence, the *IL2RA* genotype presumably affects the response of IL-2 therapy and might be relevant for personalised medicine.

Conclusion

Genetic studies are useful because they can point to mechanisms that are pivotal for disease development or disease progression. This study observed that rs2104286 in *IL2RA* and the sIL2Rα level are associated with RA persistence. Besides the *HLA-DRB1* SE, *IL2RA* is the only genetic risk factor for development of RA and for both radiographic progression and persistence. This underlines the relevance of *IL2RA* for RA. Further research is needed to gain more insight into the underlying mechanisms of arthritis persistence.

SUPPLEMENTARY DATA

Supplementary data are published on the website of *Arthritis, Research & Therapy.*

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Osteoprotegerin as biomarker for persistence of rheumatoid arthritis

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Sir, currently the treatment of Rheumatoid arthritis (RA) is aimed at achieving low disease activity score (DAS) remission. The ultimate aim, however, is to achieve disease-modifying antirheumatic drug (DMARD)-free sustained remission, which reflects the persistent absence of arthritis after cessation of treatment and is the closest proxy available for cure of RA. Although at present DMARD-free sustained remission can only be achieved in a minority of RA patients, recent data revealed that this outcome has become increasingly achievable due to improved treatment strategies [1]. The processes underlying resolution of disease persistence are unknown. An understanding of these processes might give clues for intervention targeted at disease resolutions. Furthermore, except for ACPA or RF, biomarkers for disease persistence are unknown. A recent study by Audo et al [2] prompted us to investigate the association between serum osteoprotegerin (OPG) levels and DAS remission as well as DMARD-free sustained remission. This study showed, using data of one cohort, that a low ratio of OPG to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was associated with DAS28 remission (DAS <2.6) after 1 year and that this association was completely explained by the OPG level. OPG is a member of the tumor necrosis factor (TNF) superfamily molecules. Besides its well-known role in bone metabolism, OPG has pro-inflammatory effects that likely act via the nuclear factor (NF)-kB pathway. As such, OPG has been implicated as a disease activity marker for inflammatory bowel disease [3]. The present study has two aims: first, we sought for replication of the association of OPG levels with DAS remission, as replication of findings in independent cohorts is pivotal; and second, because OPG may associate with the severity of inflammation in the short-term, we explored the association of OPG levels with achieving DMARD-free sustained remission as long-term treatment outcome.

We studied 158 RA patients (1987 ACR criteria) included in the Leiden Early Arthritis Clinic cohort 4 between 1993 and 2005 (67% female, mean (SD) age 56.2 (13.5) years, 64% ACPA-positive). OPG levels were determined using ELISA [5,6] in sera collected at a median disease duration of 4 (range 1-9) years, while patients were treated with conventional DMARDs. At the moment of serum collection, the mean (SD) DAS44 was 2.3 (1.1), 53% of patients had a DAS44 <2.4 and none of the patients were in DMARD-free sustained remission. For analyses, OPG levels were stratified in quartiles with similar patient numbers. The outcomes were achieving DAS44 remission (DAS44 <2.4 and <1.6) 1 year after serum collection and; achieving DMARD-free sustained remission during follow-up (median follow-up 10 year, IQR 9-10 years). DMARD-free sustained remission was defined as the sustained absence of arthritis (by physical examination) after discontinuation of DMARD therapy, including biologics and glucocorticosteroids, for the entire follow-up and the follow-up should be at least one year after cessation of DMARD-treatment. Patients that relapsed during follow-up were not in the DMARD-free sustained remission group. All medical files were explored on this outcome until 5 April 2012 [1]. Logistic and Cox regression analyses were performed with adjustments for age, gender and treatment strategy [4]. The analyses of DAS44 remission were additionally adjusted for DAS44 at sample collection and the analysis of

DMARD-free sustained remission were additionally adjusted for disease duration at sample collection. All patients gave informed consent and approval was obtained from the medical ethics committee of the Leiden University Medical Center.

One hundred and sixteen patients (73.4%) and 67 patients (42.4%) had achieved DAS44 remission when, defined, respectively, as DAS <2.4 and DAS <1.6 1 year after serum collection. Per quartile decrease in OPG the odds ratio (OR) for achieving DAS44 remission during the next year was 1.65 (95% CI 1.11 to 2.47, p=0.014) when defining DAS44 remission as a DAS <2.4. Similar results were obtained when defining DAS44 remission as a DAS <1.6 during the next year (OR=1.55, 95% CI 1.084 to 2.22, p=0.016). Sixteen patients (10.1%) achieved DMARD-free sustained remission after a median of 6 years of disease (IQR 4-8) and 3 years (IQR 1-4) after serum collection. Lower OPG level associated significantly with a higher chance of DMARD-free sustained remission (hazard ratio on remission per quartile decrease in OPG level 1.92 (95% CI 1.043 to 3.52, p=0.036) (Figure 1).

In conclusion, we here replicated the finding that low OPG levels were predictive for an increased chance of DAS remission during the next year. The findings were similar when using DAS remission defined as DAS44 <2.4 or <1.6. These validated results suggest that OPG is a biomarker that might be useful to assess during treatment in order to predict the chance of a low disease activity during the next year. Interestingly, serum OPG levels were also associated with the chance of DMARD-free sustained remission. Together these data suggest that OPG levels are reflective of a process influencing the severity of inflammation both on the short and long-term. Intriguingly, OPG levels did not correlate with DAS remission at the same point in time. This may suggest that the change in OPG level precede the change in inflammation that is measured by the DAS. Longitudinal studies are needed to explore this. In addition further studies are needed to confirm the association between OPG levels and DMARD-free sustained remission and to investigate the mechanisms underlying this association. Because it is likely that achieving DMARD-free sustained remission will become a preferred treatment goal in the future, further studies are also required to examine whether serum OPG levels are useful to guide treatment decisions and to predict if this favourable disease outcome is achievable.

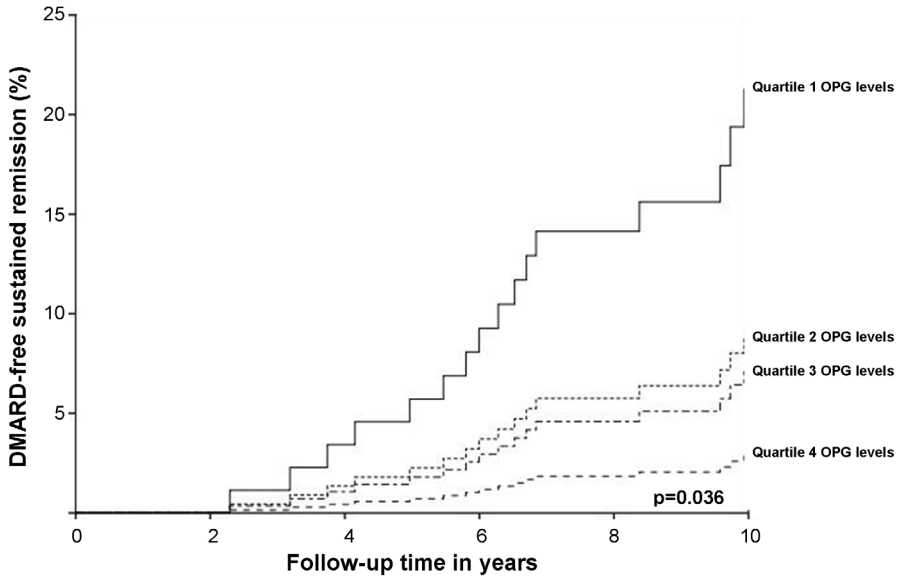

Figure 1. Osteoprotegerin levels in relation to achieving DMARD-free sustained remission in 158 rheumatoid arthritis patients. Depicted are the modeled (by the Cox regression analysis) percentages of 158 rheumatoid arthritis patients of the Leiden Early Arthritis Clinic cohort that achieved DMARD-free sustained remission during 10 years follow-up. Quartile 1 presents the lowest OPG levels and quartile 4 the highest level. The hazard ratio on achieving DMARD-free sustained remission was 1.92 (95% CI 1.043 to 3.52) per quartile decrease in OPG level (p=0.036). Quartile 1, 2, 3 and 4 concerned respectively 39, 40, 40 and 39 patients.

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Part III

Other outcomes in rheumatoid arthritis

DMARD-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms

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Sofia Ajeganova, Hanna van Steenbergen, Jessica van Nies, Leonie Burgers, Tom Huizinga, Annette van der Helm-van Mil

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ABSTRACT

Objective

Disease-modifying antirheumatic drug (DMARD)-free sustained remission, the sustained absence of synovitis after cessation of DMARD therapy, is a relevant long-term outcome of rheumatoid arthritis (RA) if (1) its occurrence is promoted by treatment and (2) this status reflects resolution of symptoms and disability. This study investigated both items.

Methods

1,007 patients with RA diagnosed between 1993 and 2011, included in the Leiden Early Arthritis Clinic, were studied on achieving DMARD-free sustained remission. Patients included in 1993–1995 were initially treated with nonsteroidal anti-inflammatory drugs, in 1996–1998 mild DMARDs were started early, from 1999 onwards methotrexate was initiated promptly and from 2005 onwards disease activity score (DAS)-steered treatment was common. Remission rates were compared using Kaplan-Meier curves and Cox proportional regression.

Results

In total, 155 patients achieved DMARD-free sustained remission. Specific treatment strategies were significantly associated with achieving remission ($p<0.001$). Cox regression adjusted for anti-citrullinated peptode antibodies/rheumatoid factor, swollen joint count, erythrocyte sedimentation rate and C-reactive protein revealed HRs for DMARD-free sustained remission of 1.13 (95% CI 0.48 to 2.64) in patients diagnosed in 1996-1998, 2.39 (1.07 to 5.32) in patients treated with early methotrexate (inclusion 1999-2004) and 3.72 (1.60 to 8.62) in those treated early with methotrexate and DAS-steered therapy (inclusion 2005-2011). At the time of remission, the Health Assessment Questionnaire was at the level of the general population (median 0.13, IQR 0–0.63). Also, patient-rated visual analogue scale (VAS) morning stiffness, fatigue, pain and disease activity were low (median (IQR) mm, 14 (2–27), 10 (0–47), 6 (0–20), 7 (0–20), respectively).

Conclusions

More intensive treatment strategies increased the chance for DMARD-free sustained remission, indicating that RA chronicity can be influenced. Patients with RA achieving DMARD-free sustained remission have a normalised functional status.

INTRODUCTION

The disease prospects of patients newly diagnosed with rheumatoid arthritis (RA) today are much better than they were decades ago [1]. The severity of joint destruction has decreased during the last years, and nowadays, clinically relevant joint destruction has become infrequent in Western countries. These advances are the result of several changes in treatment strategies. Whereas in the early 1990s disease-modifying antirheumatic drugs (DMARDs) were commenced within two years after symptom onset, DMARDs are according to current guidelines started directly after the diagnosis RA is made. Second, more potent DMARDs have been introduced as first-line therapy and biologics have become available. Finally, during the last 10 years disease activity-guided treatment adjustments have become common.

Since joint destruction has become a less relevant long-term disease outcome, other long-term out-comes will become more important. DMARD-free sustained remission is defined as the absence of sustained synovitis after cessation of DMARD therapy and is an interesting long-term outcome as it reflects loss of arthritis persistence. Because this definition intends to reflect a final disease outcome, it is different from remission outcomes that are assessed to measure treatment efficacy (for instance, clinical remission or low-disease activity). Observational studies and clinical trials have reported that DMARD-free sustained remission can be achieved in approximately 10-15% of the patients with RA [2–6].

Several questions regarding the outcome DMARD-free sustained remission remain to be answered. First, it is yet unknown whether this disease outcome is modifiable with antirheumatic treatment. We hypothesised that disease persistence (measured by its counterpart DMARD-free sustained remission) is influenced by treatment. To study this, we evaluated if patients that were treated with current treatment regimens achieve DMARD-free sustained remission more often than patients that were treated one or two decennia ago. If no difference could be observed, DMARD-free sustained remission is not a relevant long-term treatment goal; however, if up-to-date treatment strategies increase the occurrence of DMARD-free sustained remission, it is an important outcome to pursue. Second, the definition of DMARD-free sustained remission is based on joint swelling exclusively (that requires being persistently absent). In order to explore the quality of this disease outcome for patients, we studied the functional status and patient-reported symptoms such as pain and morning stiffness at the time when DMARD-free sustained remission was achieved. Finally, we answered the question whether variation in time after diagnosis until achieving DMARD-free sustained remission might reflect different patient subsets. We hence evaluated if patients that achieved DMARD-free sustained remission early, intermediate or late in disease course differed in clinical, functional or patient-reported symptoms.

PATIENTS AND METHODS

Patient population

Patients studied were included consecutively in the Leiden Early arthritis Clinic (EAC) between February 1993 and April 2011. The EAC is described elsewhere in detail [7]. In short, inclusion required the presence of confirmed synovitis and a symptom duration <2 years. At baseline and yearly follow-up visits, questionnaires were filled, joint counts performed and laboratory investigations made.

From the total cohort (n=2,731), patients that fulfilled the 1987 American College of Rheumatology criteria for RA [8] during the first year were selected (n=1,007). Patients with RA were treated according to routine care and the rheumatologists' judgements. The treatment strategies that were usually applied changed over time. Generally, patients included in 1993-1995 were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and DMARDs were started with delay, patients included in 1996-1998 were treated with mild DMARDs (mainly hydroxychloroquine or sulphasalazine) promptly after diagnosis and patients included from 1999 onwards were treated initially with methotrexate. Disease activity score (DAS)-steered treatment adjustments became common from 2005 onwards. These inclusion periods were used as a proxy for the changes in treatment strategies.

DMARD-free sustained remission

The outcome DMARD-free sustained remission was defined as the sustained absence of synovitis (by physical examination) after discontinuation of DMARD therapy (including biologics and systemic or intra-articular corticosteroids) for the entire follow-up and at least for one year after DMARD withdrawal [4,9]. This stringent definition of remission is the opposite of disease persistence. All medical files were explored on this outcome until 5 April 2012. Patients that had achieved DMARD-free sustained remission but relapsed later on (n=3) were excluded (figure 1).

The time till achieving DMARD-free sustained remission was rather arbitrarily divided into three groups: the 'early remission' group, when remission was achieved within 3 years after baseline, the 'intermediate remission' group, when remission was achieved between 3 and 5 years after baseline, and the 'late remission' group, when remission was obtained after 5–13 years.

Statistical analysis

The χ^2, t test, one-way analysis of variance and Kruskal-Wallis tests for group comparisons were used. Rates of achieving DMARD-free sustained remission were evaluated using Kaplan-Meier analysis with period of inclusion entered as grouping factor. The date of remission was defined as one year after the date at which DMARDs were withdrawn due to remission of disease. Time to remission was measured as the time from date of inclusion in the cohort to the date of remission. Cases that did not achieve remission were right-censored by their last

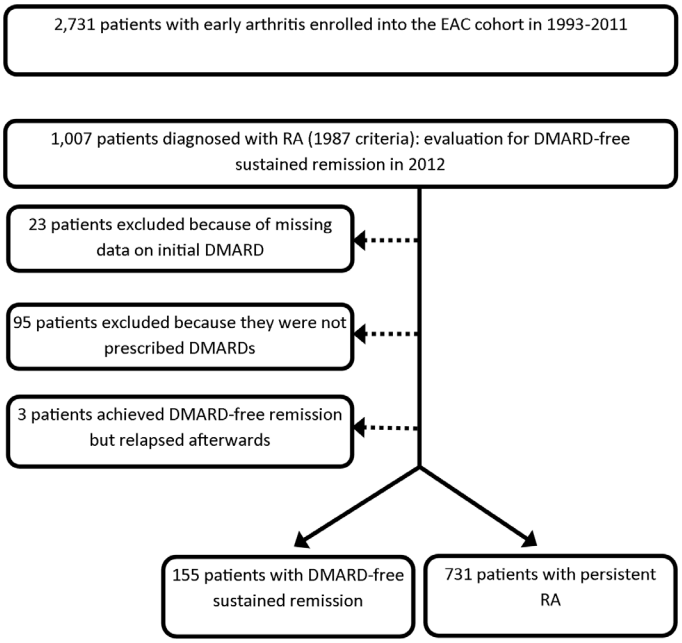

Figure 1. Flow diagram of the selection of the study participants.

known date of assessment. Equality of time-to-event function between the groups was tested with log-rank test. Cox proportional hazards regression models were used incorporating adjustments for the anti-citrullinated peptide antibody (ACPA)/rheumatoid factor (RF) status and measures of inflammation (swollen joint count (SJC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)).

A multivariate normal regression was used to depict the course of several features over time; it was not intended to test for statistical significances. Because Health Assessment Questionnaire (HAQ) and patient-assessed outcomes are known to vary with age and to be different for men and women [10,11], adjustments were made for age and gender. The multivariate normal regression takes advantage of within-patient correlation in repeated measurements and can handle missing data. The remission group, effect of time and interaction term between these variables were entered as categorical variables into the model. The best-fit covariance matrix (unstructured) was chosen according to the smaller value of the Akaike information criterion. Analyses were performed with the IBM SPSS, V.22 (SPSS, Chicago Illinois, USA).

RESULTS

Frequency of DMARD-free sustained remission over time

Table 1 presents the baseline characteristics of the 155 patients with RA who achieved DMARD-free sustained remission and the 731 patients who did not achieve it. In line with what has previously been described [4], patients who achieved DMARD-free sustained

Table 1. Baseline characteristics of the patients with RA in the study in total and according to achieved DMARD-free sustained remission or not during the follow-up

Characteristic	Total (n=886)	Remission group (n=155)	No remission group (n=731)
Age (years), mean (SD)	56.5 (15.6)	57.4 (16.4)	56.3 (15.5)
Females, n (%)	591 (67)	97 (63)	494 (68)
Symptom duration (months), med (IQR)	4.4 (2.3-8.4)	2.9 (1.8-6.5)**	4.7 (2.4-8.6)**
Smoking ever, n (%)	437 (56)	78 (54)	359 (56)
Period of inclusion, n (%)			
1993-1995	63 (7)	7 (5)	56 (8)
1996-1998	158 (18)	24 (15)	134 (18)
1999-2004	289 (33)	66 (43)	223 (31)
2005-2011	376 (42)	58 (37)	318 (43)
ACPA-positive, n (%)	464 (54)	26 (18)**	438 (62)**
ACPA low-positive†, n (%)	37 (8)	2 (8)	35 (8)
ACPA high-positive†, n (%)	427 (92)	24 (92)	403 (92)
RF-positive, n (%)	517 (59)	48 (31)**	469 (65)**
Disease markers			
ESR (mm/h), med (IQR)	32 (17-51)	29 (16-48)	32 (18-53)
CRP (mg/L), med (IQR)	15 (6-37)	16 (16-33)	15 (6-38)
SJC (0-66), med (IQR)	8 (4-13)	8 (4-15)	8 (4-13)
TJC (0-68) , med (IQR)	7 (5-11)	8 (5-11)	7 (5-11)
HAQ (0-3) , med (IQR)	1.0 (0.63-1.50)	1.0 (0.62-1.50)	1.0 (0.63-1.50)
Patient-assessed disease activity (VAS 0-100 mm), med (IQR)	54 (34-75)	51 (33-67)*	55 (34-76)*
Pain (VAS 0-100 mm), med (IQR)	51 (34-69)	48 (29-65)	52 (34-70)
Fatigue (VAS 0-100 mm), med (IQR)	48 (16-69)	40 (12-60)*	50 (17-70)*
Morning stiffness (VAS 0-100 mm), med (IQR)	63 (36-80)	57 (36-76)	64 (36-81)

*p<0.05, **p<0.001 for between-group
†ACPA low-positive refers to values that are higher than the ULN but ≤3 times the ULN for the assay, high-positive that are >3 times the ULN

remission, compared with those who did not, were less often ACPA or RF-positive (18% vs 62%, and 31% vs 65%, both p<0.001) and had shorter symptom duration at inclusion (median (IQR) of 3 months (2–7) vs 5 months (2–9), p<0.001). Patients who achieved remission, compared with those who still had persistent RA through the study period, did not have milder disease characteristics at baseline in terms of SJC, tender joint count (TJC), ESR, CRP, HAQ, morning stiffness, fatigue and frequency of high titre of ACPA.

Subsequently, the patients with RA were split into the four inclusion periods. Patients included in the more recent inclusion periods were less often ACPA-positive, had lower SJC and lower levels of acute phase reactants but they had more pain and morning stiffness at the first visit (table 2). Of these statistical differences, the difference in SJC was mostly clinically

Table 2. Baseline characteristics of the patients with RA according to period of inclusion

Characteristic	Period of inclusion				
	1993-1995 (n=63)	**1996-1998 (n=158)**	**1999-2004 (n=289)**	**2005-2011 (n=376)**	**p-value**
Age (years), mean (SD)	54.8 (14.7)	55.5 (16.9)	57.2 (15.0)	56.7 (15.8)	0.55
Females, n (%)	46 (73)	103 (65)	194 (67)	248 (66)	0.70
Symptom duration (months), med (IQR)	5.8 (3.1-11.4)	4.2 (2.0-6.9)	4.5 (2.4-9.0)	4.2 (2.1-7.5)	0.042
Smoking ever, n (%)	32 (56)	67 (47)	125 (49)	213 (65)	<0.001
ACPA-positive, n (%):	49 (78)	89 (56)	145 (52)	181 (51)	0.001
ACPA low-positive, n (%)	5 (10)	8 (9)	12 (8)	12 (7)	0.82
ACPA high-positive, n (%)	44 (90)	81 (91)	133 (92)	169 (93)	-
RF-positive, n (%)	42 (67)	89 (56)	174 (60)	212 (57)	0.45
ESR (mm/h), med (IQR)	44 (25-74)	37 (20-57)	32 (20-52)	29 (11-44)	0.13
CRP (mg/L), med (IQR)	21 (11-43)	20(9–48)	15 (7-39)	12 (4-28)	0.92
SJC (0–66), med (IQR)	8 (5-15)	12 (6.5–20)	8 (4-14)	6 (3-11)	<0.001
HAQ (0–3), med (IQR)	1.0 (0.53-1.34)	1.0 (0.62-1.47)	1.0 (0.53-1.50)	1.0 (0.63-1.50)	0.52
Patient-assessed disease activity (VAS 0-100 mm), med (IQR)	51 (28-73)	47 (25-63)	53 (39-74)	60 (36-76)	0.001
Pain (VAS 0-100 mm), med (IQR)	47 (23-60)	45 (24-59)	52 (35-67)	56 (38-71)	<0.001
Fatigue (VAS 0-100 mm), med (IQR)	38 (12-54)	32 (12-64)	49 (20-69)	50 (15-70)	0.12
Morning stiffness (VAS 0-100 mm), med (IQR)	63 (28-77)	50 (23-76)	64(39-82)	66 (40-80)	<0.009

P-values are for overall comparison of the characteristics between the four groups by period of inclusion

relevant (the median SJC of 12 in 1996-1998 and of 6 in 2005-2011). Distribution of high and low titres of ACPA was equal in the inclusion periods. Figure 2 presents the Kaplan-Meier estimates of the percentages of patients achieving DMARD-free sustained remission stratified by periods of inclusion. Patients that were treated according to more recent treatment strategies achieved remission more often (p<0.001). The mean time to remission (SD) was 7.8 (3.4), 6.9 (4.0), 4.8 (2.7) and 3.1 (1.4) years for patients treated with the subsequent treatment strategies.

To determine whether shorter follow-up for part of the patients in the last inclusion period and difference in follow-up time in different inclusion periods had influenced the results, the Kaplan-Meier analyses were repeated (1) limiting the latest inclusion period till January 2009, thus allowing for duration of follow-up of at least 3 years, and (2) limiting the maximal follow-up to 5 years. The difference between the treatment groups remained statistically significant (both analyses p<0.001, see online supplementary figure S1). The Kaplan-Meier analyses were also repeated in the ACPA-positive and ACPA-negative subgroups, both showing significant results (p=0.001 and 0.014, respectively), but DMARD-

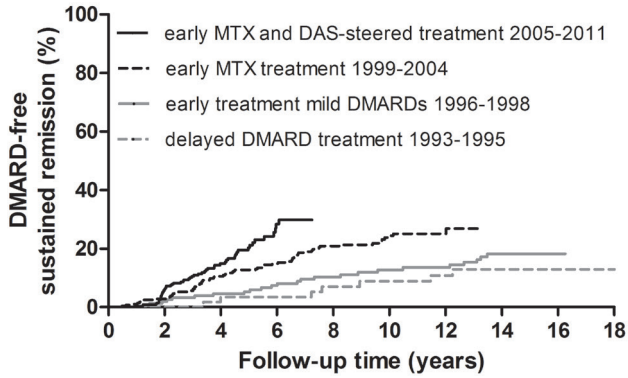

Figure 2. Kaplan-Meier estimates of the percentages of patients with RA achieving DMARD-free sustained remission, stratified for the different treatment strategies. The periods of inclusion were used as proxy for the differences in treatment strategy. Patients included in 1993-1995 were initially treated with NSAIDs and DMARDs were initiated with delay, patients included in 1996-1998 were treated early with mild DMARDs (mainly hydroxychloroquine or sulphasalazine) and patients included from 1999 onwards were promptly treated with methotrexate and DAS-steered treatment adjustments became common from 2005 onwards. Pooled over strata p<0.001 by log-rank test, test for trend p<0.001. Remission was achieved in 7 patients out of 63 patients included in 1993-1995, in 24 out of 158 patients included in 1996- 1998, in 66 out of 289 patients included in 1999-2004 and in 58 out of 376 patients included in 2005-2011.

free remission was more frequent in the ACPA-negative groups (online supplementary figure S2).

In Cox proportional hazards regression, the association between achieving remission and treatment strategy was confirmed; with the inclusion period 1993-1995 (initial treatment with NSAIDs) as reference, the HRs (95% CI) for the patients initially treated with mild DMARDs (inclusion 1996-1998), initially treated with methotrexate (1999-2004) and then with methotrexate and DAS-steered treatment (2005-2011), were 1.5 (0.6 to 3.4), 2.9 (1.3 to 6.4) and 5.3 (2.3 to 12.1), respectively. After adjustment for autoantibody status, baseline SJC, ESR and CRP the HRs were 1.13 (0.48 to 2.64), 2.39 (1.07 to 5.32) and 3.72 (1.60 to 8.62), respectively, showing an increased chance for DMARD-free sustained remission in patients treated according to more recent strategies.

Disease symptoms and functional status at the time of achieving DMARD-free sustained remission

The definition of DMARD-free sustained remission is based on the absence of swollen joints; other clinical measures and functional and patient-reported symptoms are not included in the definition. We therefore explored other disease-associated outcomes at the time of achieving remission. Of all 155 patients with remission, information on follow-up visits was missing in 48%. The missing data were completely not at random as patients with DMARD-free sustained remission declined their visit to the research nurse more often than patients with RA with persistent disease. Patients with complete data and with missing data at the time when DMARD-free sustained remission was achieved did not differ statistically significant

by age, sex, symptom duration, year of inclusion and baseline disease characteristics, except for patients' rated disease activity (median visual analogue scale (VAS) (mm) in patients with and without missing data 53 (36–72) and 47 (31–61), p=0.032). Obtained data were studied and missing data were not imputed.

The median TJC at the time of remission was 0 (IQR 0–1) and at a group level acute phase reactants were within normal limits (median (IQR) ESR 8 (5–11) mm/h and CRP 3 (3–5) mg/L). Regarding functional ability, we observed that the patients that achieved DMARD-free sustained remission had a median HAQ at diagnosis of 1.0 (IQR 0.62–1.50) (table 1) and at the time of remission of 0.13 (IQR 0–0.63) (table 3). The VAS fatigue median was 40 (12–60) mm at baseline but 10 (IQR 0–47) at the time DMARD-free sustained remission was obtained. Also, the VAS pain was low at the time of DMARD-free sustained remission, median 6 (IQR 0–20), while it was 48 (29–65) at baseline. The VAS morning stiffness decreased also to low levels in the patients that achieved remission; its median was 57 (36–76) mm at baseline and 14 (2–27) at the time of remission (table 3).

Analyses in patients with RA that achieved DMARD-free sustained remission early, intermediate or late in disease course

It was observed that the time till DMARD-free sustained remission varied between the patients. Theoretically, the rapidity with which DMARD-free remission is achieved may reflect differences in patient subsets. To explore this, characteristics of the patients that achieved DMARD-free sustained remission early (within 3 years), intermediate (3-5 years of disease) or late (5-13 years) in disease course were compared. No differences were observed either at baseline (online supplementary table S1) or at the time of achieved DMARD-free sustained remission (table 3). In order to illustrate the time course of HAQ and patient-reported symptoms in patients that achieved DMARD-free sustained remission early, intermediate and late in disease course, the median values as predicted by normal regression with adjustments for age and gender were plotted (figure 3). As a reference, the course of these variables for patients with persistent RA was plotted. The course of the CRP, ESR and TJC over time was depicted likewise (online supplementary figure S3). In all patients, the largest improvement was shown in the first year. Better outcomes across all measures were observed in the patients who achieved remission compared with those who did not.

DISCUSSION

This study explored the relevance of achieving DMARD-free sustained remission in two domains. Because DMARD-free sustained remission is generally considered to be an outcome that is obtained in only a minority of patients with RA and that the persistent nature of RA cannot be modified, the first aim was to evaluate whether the incidence of DMARD-free sustained remission changed with the use of up-to-date treatment strategies. The present data revealed indeed an increase in chance for DMARD-free sustained remission when

Table 3. Disease characteristics of the patients with RA at the time of achieved DMARD-free sustained remission in total and by groups according to time to remission after inclusion.

		Remission achieved in disease course		
Characteristic	**Total (n=155)**	**Early remission (n=47)**	**Intermediate remission (n=58)**	**Late remission (n=50)**
Age (years)	62.1 (16.5)	58.8 (17.7)	63.7 (17.3)	63.1 (14.0)
ESR (mm/h), med (IQR)	8 (5-11)	8 (4-12)	9 (5-17)	6 (4-9)
CRP (mg/L), med (IQR)	3 (3-5)	3 (3-5)	3 (3-4)	4 (3-7)
TJC (0-68), med (IQR)	0 (0-1)	1 (0-2)	0 (0-1)	1 (0-3)
HAQ (0-3), med (IQR)	0.13 (0-0.63)	0 (0-0.38)	0.25 (0-0.63)	0.25 (0.10-1.50)
Patient-assessed disease activity (VAS 0-100 mm)	7(0-20)	9 (0-21)	6 (1-14)	7 (0-26)
Pain (VAS 0-100 mm), med (IQR)	6 (0-20)	4 (0-20)	8 (0-19)	4 (0-33)
Fatigue (VAS 0-100 mm), med (IQR)	10 (0-47)	3 (0-35)	21 (2-50)	13 (1-49)
Morning stiffness (VAS 0-100 mm), med (IQR)	14 (2-27)	2 (0-32)	15 (3-20)	16 (2-53)

The data presented for the total remission group, early (remission achieved <3 years after baseline), intermediate (3–5 years after baseline) and late remission (>5–13 years) groups are based on the following number of patients: for ESR 61, 26, 24 and 11, respectively; for CRP 39, 17, 12, 10; for TJC 56, 20, 24, 12; for HAQ 60, 23, 27, 10; VAS patient-assessed disease activity and pain 71, 28, 30, 13; VAS fatigue 61, 27, 22, 13; and VAS morning stiffness 49, 19, 20 and 10.

patients were treated according to early and more intensive strategies in standard clinical practice. These findings are relevant as these data imply that also persistence of inflammation (which is the second hallmark of RA next to joint destruction) can be influenced. Significant differences were seen for the two most recent inclusion periods 1999-2011. Whether DAS-steered treatment is of additional value compared with initial methotrexate alone cannot be definitely concluded based on the present data as the follow-up duration of the inclusion period 2005-2011 was shorter than that of the other inclusion periods. But prompt treatment with methotrexate is clearly beneficial.

As DMARD-free sustained remission is defined by the findings of rheumatologists at physical examination, second, we evaluated whether this disease outcome is also relevant from the patient perspective. It was observed that when DMARD-free sustained remission was achieved the median HAQ score was 0.13. The mean HAQ for the normal population is reported to be 0.25 [12]; this suggests that the functional ability at the time DMARD-free sustained remission is achieved is normalised. Several patient-reported symptoms were studied. The VAS pain on a 100 mm scale in the general population is reported to be 11.5 and the VAS fatigue 20.5. Also, here the VAS scores at the time of DMARD-free remission (6 and 10, respectively) were lower than the reference values [13–16], suggesting that important RA-related symptoms as pain and fatigue have resolved. Together, these observations indicate that DMARD-free sustained remission is a disease outcome reflecting health state close to expected in the general population with regard to functioning and several RA-related

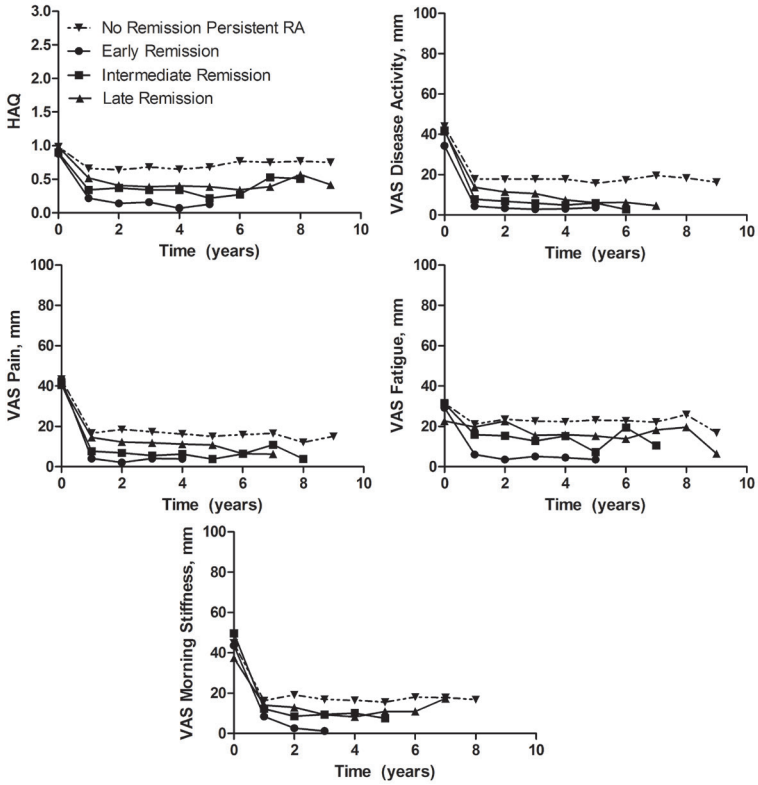

Figure 3. The course of patient-reported disease features by time to achieved remission early, intermediate and late in disease course. Presented are predicted median values obtained by the multivariate normal regression model adjusted for age at inclusion and gender. Patients that achieved DMARD-free sustained remission within 3 years after inclusion, after 3-5 years or between 5 and 13 years were referred to as early, intermediate and late remission. The lines representing the remission subgroups terminate at follow-ups with available data. As reference, the predicted values for the group of patients that did not achieve remission (dotted lines) are also shown.

symptoms.

DMARD-free sustained remission was defined as the absence of synovitis after DMARD cessation during the total follow-up that should be at least one year. The follow-up duration after DMARD cessation may potentially influence the outcome. Patients that have 1 or 2 years' follow-up after DMARD cessation might flare later on while they are now classified as being in DMARD-free sustained remission, whereas patients with a longer follow-up after DMARD cessation and who flared after several years of remission are not in the DMARD-free remission group. This might be an issue for the fourth inclusion group; all other inclusion groups were followed for many years. In a subanalysis, patients included after 2009 were excluded and only patients included in 2004-2009 were studied, thus allowing three follow-up years for all patients. The results were unchanged, suggesting a minor influence of the shorter follow-up of the fourth inclusion group.

Patients included in the more recent inclusion groups were less often ACPA-positive,

the number of swollen joints fewer and acute phase reactants lower. Although all patients studied fulfilled the 1987 criteria for RA, this may suggest that these patients had somewhat milder disease at the time of diagnosis. Also when adjustments were made for these baseline differences, patients treated according to recent protocols achieved DMARD-free sustained remission significantly more often. The finding of changed RA over time with better clinical status in contrast to worse reported subjective disease measures has been reported before [17]. This presumably reflects a change towards diagnosing (and treating) RA in a milder disease phase in recent years and higher patients' expectation of 'well-being' status [18].

An optimal study design to determine the effect of treatment strategies on DMARD-free sustained remission is a randomised clinical trial. However, at present, it will not be considered ethical to perform a trial with study arms using outdated treatment strategies. We used the longitudinal data of our observational cohort in which patients were treated according to the rheumatologists' expertise. We used the different time periods as proxies for different treatment strategies and the oldest inclusion group as reference.

It is known that the symptom duration at treatment initiation importantly affects the chance for DMARD-free sustained remission [19]. There were no large differences in symptoms duration between the inclusion periods (table 2), and when symptom duration was added as adjustment factor, the HRs were mostly unchanged (data not shown). Hence, symptom duration did not confound the results.

The biological mechanisms underlying RA persistence are unknown. We questioned whether patients that achieved DMARD-free sustained remission early in the disease constitute a different subset of patients than those that achieve it later on. We compared patient characteristics and ACPA titres between these groups at baseline and at the time of DMARD-free sustained remission and observed no relevant differences. DMARD-free sustained remission was also achieved in patients that carry autoantibodies. In total, 31% of the patients with DMARD-free sustained remission were RF-positive and 18% were ACPA-positive. Thus, the presence of autoantibodies does not absolutely impede achievement of this outcome. Exploratory analyses within ACPA-positive and ACPA-negative subgroups showed significant differences for the treatment strategies (online supplementary figure S2). Although RA persistence is more frequent in ACPA-positive RA, the present data suggest that treatment affects RA persistence in both ACPA-negative and ACPA-positive RA. More detailed studies on these subgroups are needed.

Presumably the mechanisms that promote the development of DMARD-free sustained remission, and thus the loss of RA persistence, are different from the mechanisms mediating treatment response. In this light it is interesting to note that the predictors of DMARD-free sustained remission (ACPA, symptom duration) are different from previously reported predictors for DAS response (male gender, older age, lower body mass index, lower baseline disease activity) [20–22].

The major limitation of our study is that a proportion of patients with DMARD-free sustained remission declined their research visits during remission (they were seen by rheumatologists only). 'Feeling well' might be causative for this. If this would have introduced bias, the results obtained are likely derived from the worst selection of patients with DMARD-free sustained remission because the patients who had the best physical function or the least symptoms in particular refused further study participation. We did not observe large differences in baseline characteristics between DMARD-free remission patients with and without data at the time of remission.

In conclusion, RA can no longer be considered as an inevitably chronic disease. The results of the present study indicate that DMARD-free sustained remission is increasingly achievable in the recent years of early and intensified antirheumatic therapy. Therefore, DMARD-free sustained remission is an advantageous long-term disease outcome that is relevant to pursue.

SUPPLEMENTARY DATA

Supplementary data are published on the website of the *Annals of the Rheumatic Diseases.*

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Fatigue in rheumatoid arthritis; a persistent problem: a large longitudinal study

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ABSTRACT

Objective

Fatigue is prevalent and disabling in rheumatoid arthritis (RA). Surprisingly, the long-term course of fatigue is studied seldom and it is unclear to what extent it is influenced by inflammation. This study aimed to determine the course of fatigue during 8 years follow-up, its association with the severity of inflammation and the effect of improved treatment strategies.

Methods

626 patients with RA included in the Leiden Early Arthritis Clinic cohort were studied during 8 years. Fatigue severity, measured on a 0–100 mm scale, and other clinical variables were assessed yearly. Patients included in 1993–1995, 1996–1998 and 1999–2007 were treated with delayed treatment with disease-modifying antirheumatic drugs (DMARDs), early treatment with mild DMARDs and early treatment with methotrexate respectively. After multiple imputation, the serial measurements were analysed using linear quantile mixed models.

Results

Median fatigue severity at baseline was 45 mm and remained, despite treatment, rather stable thereafter. Female gender (effect size=4.4 mm), younger age (0.2 mm less fatigue per year), higher swollen and tender joint counts (0.3 mm and 1.0 mm more fatigue per swollen or tender joint) and C reactive protein-levels (0.1 mm more fatigue per mg/L) were independently and significantly ($p<0.05$) associated with fatigue severity over 8 years. Although improved treatment strategies associated with less severe radiographic progression, there was no effect on fatigue severity ($p=0.96$).

Conclusions

This largest longitudinal study on fatigue so far demonstrated that the association between inflammation and fatigue is statistically significant but effect sizes are small, suggesting that non-inflammatory pathways mediate fatigue as well. Improved treatment strategies did not result in less severe fatigue. Therefore, fatigue in RA remains an 'unmet need'.

INTRODUCTION

Fatigue is common in rheumatoid arthritis (RA) and considered as one of the most important disease outcomes by patients [1]. Although fatigue is not clearly defined and the cut-off for clinically relevant fatigue of most fatigue measures is unknown, the prevalence of fatigue in RA has been reported to be 40–80% [2,3]. The majority of studies evaluating fatigue in RA had cross-sectional designs or studied patients with RA with different disease durations; therefore little is known on the long-term course of fatigue [4].

The importance of fatigue in RA is underlined by the finding that more severe fatigue is predictive for decreased physical and mental health-related quality of life, depression and loss of work ability [5–7]. Both a Patient Perspective Workshop at OMERACT (Outcome Measures in Rheumatology) in 2007 and a EULAR/ACR task force in 2008 recommended that 'each trial should report on fatigue' [8,9]. Furthermore, since severe radiographic joint destruction (the traditional outcome measure in trials) is no longer prevalent, other outcomes will become more important. In the light of these developments, it is surprising that so little is known about the fatigue severity during the disease course.

The causation of fatigue in RA is thought to be multidimensional. A recently proposed conceptual model suggests that fatigue is the result of interactions between three factors: disease-process related factors, cognitive and behavioural factors (thoughts, feelings, behaviours) and personal factors (personal life issues) [10]. Scientific data supporting this model are mostly derived from cross-sectional studies and are conflicting [4]. A recent systematic literature review concluded that none of the studied associations were consistent across all published data [4]. Most consistent were the associations of fatigue severity with the degree of pain and physical impairments. To a lesser extent, there was some consistency in the results showing that depressive mood/depression and female sex associated with more severe fatigue [2,4,11–15]. In contrast, the relation between markers of inflammation and fatigue remained disputable as contradictory results were obtained on the associations between fatigue and erythrocyte sedimentation rate, C reactive protein (CRP), joint counts and disease activity scores [2,4,11–15]. Thus, although intuitively it is generally felt that fatigue in RA is in part the consequence of inflammation (either clinically apparent or subclinical inflammation), the question whether and to what extent fatigue in RA is mediated by inflammation remains unanswered.

This study aimed to assess (1) whether fatigue severity differed at disease onset between patients presenting with RA and other forms of early arthritis. Within RA, a large longitudinal 8 years study on fatigue was performed aiming to determine (2) the course of fatigue, (3) the association between the course of inflammation and the course of fatigue and (4) the effect of improved treatment strategies, which have resulted in better suppression of disease activity, on the course of fatigue.

PATIENTS AND METHODS

Longitudinal cohort

All patients were included in the Leiden Early Arthritis Clinic cohort, a population-based inception cohort in the Netherlands that started in 1993 and has been described in detail previously [16]. In short, inclusion took place when arthritis was confirmed at physical examination and symptom duration was <2 years. At baseline, rheumatologists completed questionnaires, 66-swollen and 68-tender joint counts (SJC and TJC) were performed, patients filled out questionnaires among which the Health Assessment Questionnaire (HAQ), hand and feet radiographs were taken, as well as blood samples for routine laboratory screening (including CRP, haemoglobin (Hb), immunoglobulin (Ig)M-rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) (anti-CCP2, Eurodiagnostica, the Netherlands). Written informed consent was obtained from all patients. The study was approved by the local Medical Ethics Committee.

To measure fatigue severity, patients were asked by the research nurses to note the point that best reflected the fatigue they had experienced during the last day [17]. This was expressed on a visual analog scale (VAS) ranging from 0 (no fatigue) to 100 mm (extreme fatigue) for the patients assessed between 1993 and 2010 and on a numerical rating scale (NRS) ranging from 0 (no fatigue) to 10 (extreme fatigue) from 2010 onwards, when digital recording forms were introduced. Since the VAS and NRS are known to correlate strongly [18,19], results of the NRS were multiplied by 10.

Follow-up visits were performed yearly and included standard clinical assessment including joint counts, rating the fatigue severity, HAQ and radiographs of hands and feet. Serial radiographs were scored with known time-order by one experienced reader using Sharp-van der Heijde scores (SHS) blinded to any clinical data (intraclass correlation coefficient 0.91), as described previously [16]. The initial treatment strategy of patients with RA has changed over time. Generally, patients included in 1993–1995 were initially treated with nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs) were initiated with delay, patients included in 1996–1998 were early treated with mild DMARDs (mainly hydroxychloroquine or sulphasalazine) and patients included in 1999–2007 were early treated with methotrexate. In addition, since 1999 DMARD-therapy was more rapidly adjusted in case of insufficiently suppressed disease activity score (DAS-steered treatment).

Patient selection

Between 1993 and March 2013 in total 3,112 early patients with arthritis with different diagnoses were included in the EAC. Of these patients, 2,442 (78.5%) had baseline fatigue data. Patients with and without baseline fatigue data did not differ in age, gender, SJC, TJC, or frequency of ACPA-positivity or having RA; patients with missing baseline fatigue data had slightly higher CRP-levels (median (IQR) 12 mg/L (4–37) versus 10 mg/L (3–27), p=0.002). All 2,442 early patients with arthritis were studied to compare fatigue at disease onset across

different diagnoses.

Of the 2,442 early patients with arthritis included between 1993 and 2013, 902 patients fulfilled the 1987-ACR criteria for RA. Associations of baseline characteristics with fatigue at disease onset in RA were studied in these 902 patients.

Longitudinal studies on yearly measured fatigue were done on the 626 patients with RA included before 2007, providing the possibility to have completed 8 years follow-up. Similar as for fatigue, SJC, TJC, CRP, Hb, HAQ and SHS were measured yearly, providing the opportunity to test associations between fatigue and the concomitantly measured clinical variables during follow-up. In other words, this allowed studying the course of other variables in relation to the course of fatigue. Furthermore, in these patients the effects of improved treatment strategies in RA on three different long-term outcomes were studied, namely radiographic progression, SJC and fatigue over time.

A large majority of patients without RA (e.g. patients with reactive arthritis, inflammatory osteoarthritis or paramalignant arthritis) were not followed for many years. Patients diagnosed with undifferentiated arthritis (UA), psoriatic arthritis/spondylarthropathy with peripheral arthritis (PsA/SpA), (pseudo)gout and systemic connective tissue disease (SCTD) often did not complete 8 years follow-up but considerable proportions completed 3 years follow-up. In these patients, available fatigue data were evaluated to determine whether the 3 years course of fatigue was different in RA compared to other forms of chronic arthritis.

Statistical analyses

As fatigue severity was non-normally distributed, median levels were presented and modelled. Cross-sectional quantile regression analyses were used for the comparisons on baseline fatigue data (comparisons across different diagnoses and within RA). For the longitudinal 8-year analyses of fatigue within RA linear quantile mixed models were used [20,21] modelling the course of medians over time instead of means as in linear models [22]. SJC, TJC, CRP, Hb, SHS and HAQ were measured yearly and not constant over time and analysed for its association with fatigue over time as time-dependent variables. Age, gender, ACPA and RF reported or measured at baseline were analysed. Before performing the analyses within RA, multiple imputation was used to deal with missing data for fatigue severity, symptom duration, duration of morning stiffness, SJC, TJC, CRP, Hb, RF, ACPA, SHS and HAQ. Five datasets were created for both the analyses of associations with fatigue at disease onset within RA and for the repeated measurements over 8 years by using multivariate imputation by chained equations that generates multiple imputations for incomplete multivariate data by Gibbs sampling [23].

The association between the different treatment strategies (reflected by the proxy different inclusion period) and radiographic progression was analysed over 8 years using a multivariate normal regression analysis adjusted for age and gender, as described in detail previously [24,25]. A linear quantile mixed regression analysis was used to analyse whether

different treatment strategies associated with the number of swollen joints over 8 years.

Analyses were performed using R statistical software package [26,27] and SPSS V.20.0. P-values <0.05 were considered significant.

RESULTS

Baseline fatigue severity across different forms of early arthritis

Table 1 presents the characteristics of the studied patients. First, we studied whether the fatigue severity differed between patients presenting with RA and other forms of early arthritis. Of the 2,442 patients with fatigue data at disease onset, 902 patients had RA and 1,540 patients other diagnoses. Figure 1A presents medians of fatigue severity for different diagnoses. Patients with SCTD and RS3PE recorded significantly more severe fatigue than patients with RA (medians respectively 59 mm and 58 mm compared to 49 mm in RA; p<0.05 adjusted for age and gender). Patient groups that experienced significantly less fatigue than patients with RA were patients with UA (median 37 mm), PsA/SpA (median 30 mm) and septic arthritis (25 mm) (all p<0.05 adjusted for age and gender). Four included patients were finally diagnosed with post-traumatic joint swelling; these patients had a median fatigue severity of zero (figure 1). Evaluating available data on the fatigue course over the first 3 years of disease revealed a similar trend as for the baseline data: patients with SCTD remained more severe fatigued than patients with RA and patients with PsA/SpA and (pseudo)gout had less severe fatigue also during follow-up (figure 1B).

Baseline fatigue severity in relation to baseline characteristics in RA

Within the 902 patients with RA it was studied whether fatigue at disease onset associated with other baseline characteristics (table 2). Independent associations with more severe fatigue were observed for female (p=0.001), patients with more morning stiffness (p=0.002), more tender joints (p<0.001) and patients without ACPA (p=0.003). Also longer symptom duration at disease presentation associated with more severe fatigue at the first visit (p<0.001). Additionally, patients that reported a higher level of disability (HAQ) reported also more severe fatigue (20.5 mm increase in fatigue severity with 1 point increase in HAQ, p<0.001).

Course of fatigue in RA

626 patients with RA were studied during 8 years follow-up. At baseline, the median (IQR) fatigue severity was 45 mm (18–65) and, despite initiation of treatment, the fatigue severity slightly decreased during the first year but then remained rather stable (figure 2).

Course of fatigue in relation to course of inflammation in RA - univariable analyses

Measures of inflammation (SJC, TJC and CRP) changed during the disease course as result of either the disease itself or the applied intervention. Similar to fatigue, these markers were measured yearly, allowing determining associations between the course of inflammation and the course of fatigue during 8 years. Patients that over time remained to have higher number

Table 1. Baseline characteristics

	Patients with RA with fatigue data at disease onset (n=902)*	Non-RA early patients with arthritis with fatigue data at disease onset (n=1540)†	Patients with RA with repeated fatigue data over 8 years (n=626)‡
Age at disease onset, mean (SD), years	56.6 (15.3)	49.9 (17.4)	56.5 (15.5)
Female, n (%)	607 (67.3)	868 (56.4)	429 (68.5)
Inclusion period	1993–2013	1993–2013	1993–2007
Symptom duration, median (IQR), weeks	18.3 (9.3–34.7)	13.1 (5.1–29.1)	19.3 (10.7–39.3)
Morning stiffness, median (IQR), minutes	60 (30–120)	30 (0–60)	60 (30–120)
SJC, median (IQR)	7 (4–12)	2 (1–6)	8 (4–14)
TJC, median (IQR)	4 (7–10)	4 (2–7)	8 (5–12)
CRP, median (IQR), mg/L	14 (6–33)	8 (3–23)	17 (8–39)
Hb, mean (SD), mmol/L	8.2 (0.8)	8.4 (0.9)	8.1 (0.8)
ACPA-positive, n (%)	456 (52.1)	105 (9.1)	326 (54.1)
RF-positive, n (%)	516 (57.9)	228 (15.1)	364 (59.2)
SHS, median (IQR)	5 (2–11)	N/A	5 (2–11)
HAQ, median (IQR)	1 (0.6–1.5)	0.6 (0.3–1.0)	1 (0.6–1.5)

*Symptom duration was missing in 54 patients, morning stiffness in 78 patients, SJC in 14 patients, TJC in 236 patients, CRP in 30 patients, Hb in 18 patients, ACPA-status in 27 patients, RF-status in 11 patients, SHS in 372 patients and HAQ in 108 patients.
†Symptom duration was missing in 113 patients, morning stiffness in 188 patients, SJC is 37 patients, TJC in 467 patients, CRP in 78 patients, Hb in 43 patients, ACPA-status in 392 patients, RF-status in 31 patients and HAQ in 221 patients. The radiographs of the patients without RA were not SHS scored.
‡Symptom duration was missing in 37 patients, morning stiffness in 19 patients, SJC in 4 patients, TJC in 334 patients, CRP in 36 patients, Hb in 16 patients, ACPA-status in 23 patients, RF-status in 11 patients, SHS in 19 patients and HAQ in 104 patients. The missing values were imputed for the analyses of associations between fatigue and other variables within RA. SJC=66-swollen joint count; TJC=68-tender joint count; SHS=Sharp-van der Heijde score; HAQ=health assessment questionnaire; N/A=not applicable

of swollen joints, tender joints and CRP-levels also remained more fatigued (all $p<0.001$). The observed effect sizes of the inflammatory markers were all small. For example, presence of one additional swollen joint over time associated with 0.7 mm more fatigue over time and patients with 1 mg/L higher CRP over time reported 0.1 mm more fatigue (table 3).

Although we aimed to evaluate whether the course of inflammation associated with the course of fatigue, some other clinical variables that might be of importance when evaluating fatigue severity were studied. Female and younger patients remained more fatigued over time (females 6.1 mm more severe fatigue than males, $p<0.001$; 0.2 mm less severe fatigue per year older, $p=0.043$, table 3). The course of fatigue was similar for patients with and without RA-related autoantibodies. Patients with lower Hb-levels during the disease course remained more fatigued ($p=0.003$).

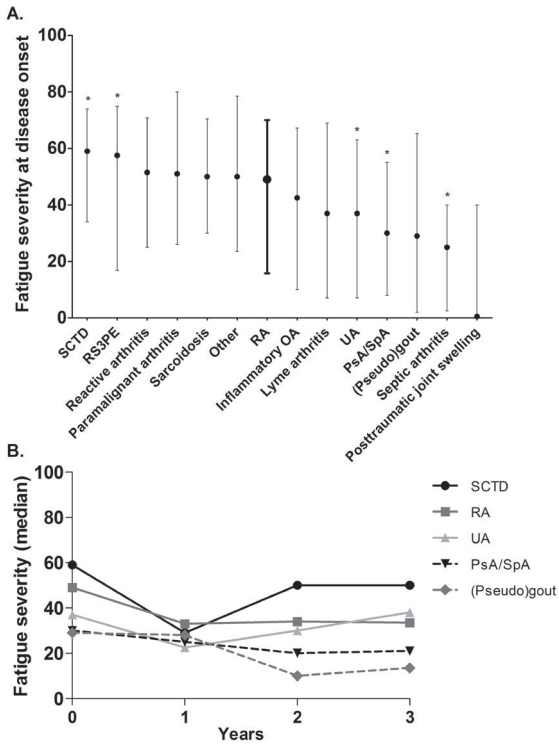

Figure 1. Fatigue severity across early patients with arthritis with different diagnoses at disease onset (A) and over 3 years of disease (B). (A) Presented are medians and IQRs of fatigue severity at disease onset. An asterisk indicates a significant different fatigue level compared to RA when adjusted for age and gender. The numbers of patients at baseline are 902 for RA, 73 for SCTD, 48 for RS3PE, 96 for reactive arthritis, 19 for paramalignant arthritis, 65 for sarcoidosis, 25 for others, 126 for inflammatory OA, 13 for lyme arthritis, 706 for UA, 271 for PsA/SpA, 90 for (pseudo) gout, four for septic arthritis and four for post-traumatic joint swelling. (B) Presented are medians of fatigue severity over 3 years of disease. Available, unmodelled data without imputation of missing data is depicted. The numbers of available fatigue data per diagnosis at baseline, one, 2 and 3 years follow-up were respectively: 73, 32, 25 and 21 for SCTD; 902, 537, 411 and 432 for RA; 706, 270, 155 and 139 for UA; 271, 151, 110, 101 for PsA/SpA; 90, 13, 4 and 2 for (pseudo)gout. SCTD=systemic connective tissue disease;

Furthermore, patients with more functional impairment during the disease course (higher HAQ-scores), were also more severe fatigued; per point increase in HAQ the fatigue severity increased with 14.6 mm (p<0.001). (Figures that present fatigue scores over time in relation to some characteristics are available from the corresponding author on request).

Course of fatigue in relation to course of inflammation in RA - multivariable analysis

Characteristics with p<0.05 in univariable analyses and clinically relevant variables were included in multivariable analysis. This revealed that higher SJC (p=0.022, 0.3 mm more fatigue per swollen joint), higher TJC (p<0.001, 1.0 mm more fatigue per tender joint) and higher CRP (p=0.049, 0.1 mm more fatigue per mg/L CRP) (table 3) associated with more severe fatigue over time, indicating a significantly independent association of the severity of inflammation over time with persistence of more severe fatigue. Also female (p=0.022,

Table 2. Fatigue severity at disease onset in relation to clinical variables at disease onset in rheumatoid arthritis

	Effect size in mm (SE)	p-value	Effect size in mm (SE)	p-value
	Univariable analyses		Multivariable analysis	
Female	7.1 (3.4)	0.040*	7.6 (2.3)	0.001*
Age at onset, per year	-0.004 (0.1)	0.95	-0.01 (0.1)	0.20
Symptom duration, per week	0.1 (0.02)	<0.001*	0.1 (0.02)	<0.001*
Morning stiffness duration, per minute	0.04 (0.01)	<0.001*	0.03 (0.01)	0.002*
SJC, per joint	0.4 (0.2)	0.010*	-0.3 (0.2)	0.071
TJC, per joint	1.4 (0.2)	<0.001*	1.1 (0.2)	<0.001*
CRP, per mg/L	0.1 (0.05)	0.072	0.1 (0.03)	0.096
Hb, per mmol/L	-3.6 (1.2)	0.002*	-0.9 (1.5)	0.54
ACPA-positivity	-4.3 (3.0)	0.15	-7.9 (2.6)	0.003*
RF-positivity	-0.3 (2.4)	0.90	3.8 (2.7)	0.15
SHS, per point	0.02 (0.2)	0.93		

*p<0.05.
Presented are the results of the analyses on 902 patients with RA with fatigue severity at disease onset as outcome and the other variable at disease onset as independent variable. Variables with p-values <0.05 in univariable analyses and clinically relevant variables were included in multivariable analysis. The effect sizes indicate how much the fatigue severity at disease onset on a 0–100 mm scale changed with 1 unit increase in the other variable at disease onset. For example, women have a 7.1 mm higher fatigue severity at baseline compared to men and the fatigue severity measured at disease onset increased 1.4 mm per tender joint.

difference with males 4.4 mm) and younger patients (p=0.016, 0.2 mm lower scores per year older) reported more severe fatigue.

Improved treatment strategies and the course of fatigue in RA

In general, improved treatment strategies in RA have resulted in improved disease outcomes. To validate this notion in present dataset, we explored the association of the described treatment strategies with the severity of radiographic progression. A significant difference in radiographic progression was observed between the three groups: with delayed DMARD-treatment as reference (inclusion 1993–1995), patients early treated with mild DMARDs (inclusion 1996–1998) had 0.97-fold less severe radiographic progression per year (p=0.026) and patients early treated with methotrexate followed by DAS-steered treatment (inclusion 1999–2007) had 0.92-fold less severe radiographic progression per year (p<0.001, figure 3A). We also evaluated whether the median number of swollen joints over time was different for the patients with RA treated with different treatment strategies. Indeed, improved treatment strategies associated with a reduction in SJC during disease course: with the delayed treatment group (1993–1995) as reference, patients early treated with mild DMARDs (1996–1998) had 1.4 less swollen joints (p=0.005) over 8 years and patients early treated with methotrexate (1999–2007) had 3.6 less swollen joints over 8 years (p<0.001) (figure 3B). In line with these observations, we hypothesised that improved treatment strategies also associated with a less severe fatigue course. However, no univariable association was observed. Patients with RA

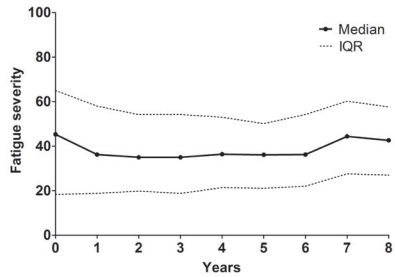

Figure 2. The severity of fatigue over 8 years of disease in early rheumatoid arthritis patients. Presented are the median values with IQR of fatigue severity in 626 early patients with RA with missing data imputed. The numbers of patients with available data per year were: 510 for baseline, 350 for year 1, 298 for year 2, 280 for year 3, 266 for year 4, 251 for year 5, 208 for year 6, 192 for year 7 and 166 for year 8.

Table 3. Fatigue severity over 8 years in relation to the course of inflammation and other variables in rheumatoid arthritis

	Effect size in mm (SE)	p-value	Effect size in mm (SE)	p-value
	Univariable analyses		Multivariable analysis	
Female	6.1 (1.7)	<0.001*	4.4 (1.9)	0.022*
Age at disease onset, per year	-0.2 (0.1)	0.043*	-0.2 (0.1)	0.016*
SJC, per joint	0.7 (0.1)	<0.001*	0.3 (0.1)	0.022*
TJC, per joint	1.1 (0.1)	<0.001*	1.0 (0.1)	<0.001*
CRP, per mg/L	0.1 (0.03)	<0.001*	0.1 (0.04)	0.049*
Hb, per mmol/L	-3.6 (1.2)	0.003*	-0.9 (1.1)	0.38
ACPA-positivity	-0.5 (2.0)	0.79	0.4 (2.1)	0.84
RF-positivity	-3.3 (1.7)	0.053	-1.9 (2.1)	0.36
SHS, per point	-0.1 (0.1)	0.14		

Presented are the results of the longitudinal analyses in 626 patients with RA. Variables with p-values <0.05 in univariable analysis and clinically relevant variables were included in multivariable analysis. The outcome (fatigue) and the clinical variables SJC, TJC, CRP, Hb and SHS were measured yearly. Gender, age, RF and ACPA were determined at disease onset. The effect sizes indicate how much the fatigue on a 0–100 mm scale change with 1 unit increase in the other variable. For example, 1 mg/L increase in CRP is associated with a 0.1 mm increase in fatigue severity measured at the same time point during 8 years follow-up and women have a 6.1 mm higher fatigue score at every time point over 8 years than men.

early treated with mild DMARDs or methotrexate did not experience less severe fatigue over time compared to patients treated with initial treatment with NSAIDs and delayed DMARD-therapy (p=0.80 and p=0.79, respectively, figure 3C). This indicates that despite improved treatment strategies and subsequent decreased inflammation-levels during the disease course, the fatigue severity in RA remained unchanged.

DISCUSSION

Although fatigue in RA is convincingly known to be associated with functional loss and is considered an important outcome measure for clinical trials [8,9], little is known on its long-

term course. Furthermore, the results of existing studies on the causation of fatigue were conflicting with regards to whether fatigue is associated with inflammatory markers. This large longitudinal study evaluated the course of fatigue in RA and observed that patients with higher inflammatory markers (more tender/swollen joints, higher CRP-level) over time also experienced more severe fatigue over time. Hence, persistence of inflammation is statistically significant associated with more severe fatigue, but the observed effect sizes were small. Furthermore, improved treatment strategies did not result in a less severe fatigue course.

Previous studies evaluating fatigue in RA observed inconsistent results on whether inflammation associated with fatigue. The majority of these studies had cross-sectional study designs or short follow-up [4] and were presumably underpowered to observe significance for small effects. Present study was powerful because of the repeated measurements during 8 years follow-up and by the use of a statistical method that takes advantage of the correlation between the serial measurements. Although we clarified that a relation between persistent inflammation and persistent fatigue is present, the question is whether the observed effect sizes are clinically relevant. The minimal clinically important difference (MCID) for the VAS fatigue, which is the smallest improvement in score that is perceived as beneficial by patients, has been reported to be around 10 mm [28] and the unadjusted effect size per swollen joint was 0.7 mm, indicating that per five additional swollen joints, fatigue severity increased with 3.5 mm on a 0–100 mm scale. Although the observed effect sizes were small, when a combination of characteristics is present, for instance several tender/swollen joints and increased CRP, the limit of a clinically meaningful difference of fatigue is reached.

Based on the observed association between inflammation and fatigue and on the efficacy of improved treatment strategies on both inflammation and radiographic progression, we hypothesised that these treatment strategies had resulted also in less severe fatigue. The inclusion periods were used as proxies for treatment strategy. A limitation is that other (unmeasured) factors changed over time as well. Nonetheless, patients with RA treated according to nowadays regimens experienced equally severe fatigue as patients initially treated with NSAIDs and initiation of DMARDs with delay. This finding might be unexpected as the latter patient group had less severe inflammation over time (for instance lower SJCs) and we had observed that less inflammation associated with less severe fatigue. Apparently other factors with contrary effects on fatigue were present as well.

We then hypothesised that patients treated with nowadays regimens and consequently with less inflammation during the disease, rated their fatigue higher and therefore the decrease in levels of inflammation was not accompanied by a decrease in fatigue severity. To gain support for this hypothesis, an additional multivariable analysis including inflammatory markers and the applied treatment strategy (inclusion period) was performed. This revealed that when adjusted for inflammatory measures, patients treated in most recent treatment group (inclusion 1999–2007) had indicated significantly more severe fatigue compared to patients included in 1993–1995 (p=0.013). Furthermore, SJC and CRP were not significantly

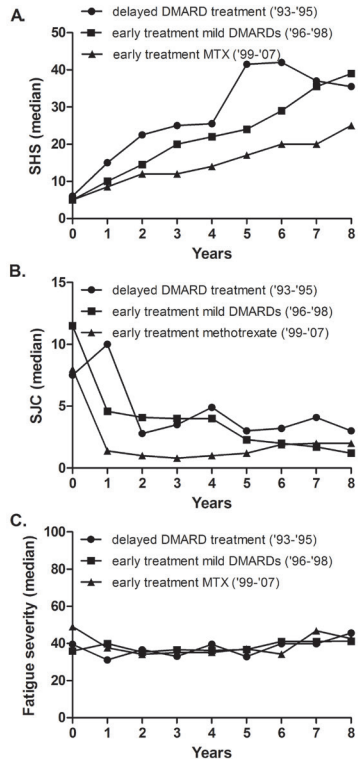

Figure 3. Different treatment strategies in rheumatoid arthritis in relation to radiographic progression (A) number of swollen joints (B) and fatigue severity over time (C). Presented are three long-term outcomes in relation to treatment strategies. Treatment strategies are reflected by different inclusion periods as the initial treatment strategy differed for different inclusion periods. The inclusion period 1993–1995 comprised 100 patients, 1996–1998 166 patients and 1999–2007 360 patients. Radiographic progression: 1993–1995=reference, 1996–1998 β=0.97 p=0.026; 1999– 2007 β=0.92 p<0.001. The β indicates the fold rate of joint destruction per year compared to the reference. Swollen joint count: 1993–1995=reference, 1996–1998 effect size=−1.4 p=0.005; 1999–2007 effect size=−3.6 p<0.001, omnibus test for overall significance of model p<0.001. The effect size indicates the difference in number of swollen joints compared to the reference. Fatigue severity: 1993–1995=reference, 1996–1998 p=0.80; 1999–2007 p=0.79; omnibus test for overall significance of model p=0.96.

associated anymore (data are available from the corresponding author on request). This result is in line with that of Putrik et al [29] who observed that patients with RA in countries with higher welfare scored worse on patients-reported outcomes like fatigue despite lower levels of inflammation. Also in line is a study that compared patients with RA in 1985 and 2000, showing that improvement in objectively measured disease outcomes was not accompanied by improvement in patient-reported pain [30]. Although causality can never be proved using association analyses, a similar effect might explain the persistence of fatigue despite decreased levels of inflammation. Present results, at least illustrate the multidimensional origin of fatigue and imply that non-inflammatory pathways are relevant as well. Consequently, other types of intervention should be initiated to reduce the disease burden caused by fatigue [31].

Notably, biologics were rarely used in the patients with RA studied here (<5%) [24];

other studies are needed to determine whether treatment with biologics differently affect fatigue severity in RA.

We observed more severe fatigue in female patients with RA. This is in line with results obtained in other studies [4]. Sex differences in RA have been reported not only for fatigue but also for other patient-reported out-comes like pain, function and global status with female having worse scores [32,33].

The fatigue data were non-normally distributed. To prevent that a small proportion of patients disproportionally affected the study results, quantile regression analyses were performed. This method models medians instead of means and is more robust to outliers. Over time patients had missing data, both on fatigue and other variables. Since these missing values were not completely at random, analyses of available data may introduce bias [34] and therefore, multiple imputation was performed. However, no large differences are seen in the 8-year course of fatigue when using available data only and after imputation of missing values (data are available from the corresponding author on request).

We performed analyses on group level. We realise that this inflicts simplicity of reality because changes of individuals are averaged. However, presumably, there are individual patients with a remarkable decrease in fatigue severity during disease course (in response to treatment or otherwise), while others may have stable or increasing levels of fatigue. To get some insight in this assumption, we evaluated fatigue severity on patient level during the first 2 years of disease. It was observed that during the first and second year of disease, respectively 49% and 62% of the patients had rather stable fatigue levels while the others had an increase or decrease in fatigue severity. Furthermore, it was observed that the patients with most severe fatigue were partly different at different time-points (data are available from the corresponding author on request). This indicates that on patient level fatigue severity can differ during the disease course and that on group level fatigue remains a persistent problem in RA.

We measured fatigue severity using a VAS. Several other fatigue measures are available; these are multi-item or multidimensional measures that explore broader fatigue issues or various domains of fatigue. Such multidimensional measures are useful for exploring fatigue causality and evaluation of fatigue interventions. Compared to such tools, a VAS provides a one-dimensional assessment of fatigue that is focused on its severity. Advantageous of the VAS is that it is simple to administer. Also the test-retest, construct validity and sensitivity to change in RA are reported to be good [17].

Present study mainly focused on the longitudinal course of fatigue in patients with RA, though we also evaluated whether fatigue severity of patients with RA differed to fatigue experienced by patients with other forms of early arthritis. Patients with SCTD and RS3PE had significantly more severe fatigue and patients with UA, PsA/SpA or septic arthritis had significantly less fatigue compared to patients with RA. However, some of the observed

differences were small and it can be questioned whether these differences are clinically relevant. The differences observed at baseline remained present over time. To the best of our best knowledge, this has not been reported on this scale previously.

To conclude, in this large longitudinal study on fatigue in RA, fatigue was a persistent problem, despite treatment. The median level of fatigue experienced by a population-based cohort of patients with RA remained even after many years of disease around 40 mm and the applied treatment strategies did not reduce fatigue levels. Therefore, as persistent fatigue is associated with functional loss, fatigue in RA remains an 'unmet need'.

SUPPLEMENTARY DATA

Supplementary data are available from the author on request.

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Summary and discussion

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In this thesis, studies were performed on the very early and early phases of rheumatoid arthritis (RA). In **Part I**, the phase of Clinically Suspect Arthralgia (CSA) was investigated. Since it became clear that early aggressive treatment of RA has much more effect in terms of preventing joint damage progression and achieving remission, a challenge in the rheumatologic field is now to identify and treat RA as soon as possible. The earliest moment to clinically recognise patients who may develop RA is the phase of CSA. In Part II and III, studies were performed within early RA. In **Part II**, genetic risk factors for a more severe disease course, mainly joint damage progression, were studied. These studies contributed to our understanding of processes that are fundamental to disease progression. **Part III** focussed on other outcomes in RA, among which patient-reported outcomes.

PART I: THE PHASE OF CLINICALLY SUSPECT ARTHRALGIA

This thesis started (**Chapter 2**) with a review on literature on the preclinical phases of RA [1]. This revealed that there is convincing evidence that autoantibody development and maturation occurs before clinically detectable arthritis develops and suggestive evidence that systemic and local inflammation are already present in this phase. RA development can thus be considered a multiple hit process in which RA-related processes can be active already years before RA is diagnosed. The studies reviewed were mainly performed in autoantibody-positive populations and we observed that studies on the preclinical and very early phases of autoantibody-negative RA were scarce. This review ended with a research agenda for studies on the very early phases of RA.

The phase of symptoms without clinically apparent arthritis is the first moment that imminent RA can be clinically recognised. Since the symptoms that are characteristic for this phase are not yet known [1], we studied this phase by investigating patients with Clinically Suspect Arthralgia (CSA). CSA was defined as arthralgia without clinically detectable arthritis that was considered by the rheumatologist clinically suspect to progress to RA and thus, the decision on whether a patient had CSA depended on the clinical expertise of the rheumatologist. In addition, the decision on the presence of CSA was made at the first visit before additional tests were performed and thus did not depend on the autoantibody-status of the patient. This concept differed from that used in other studies focusing on this symptomatic phase that studied autoantibody-positive patients with unspecified arthralgia or non-specific musculoskeletal symptoms [2–4]. The advantage of the CSA approach is that it is in line with the clinical practice where patients present with certain symptoms and the decision to perform additional investigations is based on the clinical presentation. Furthermore, it allows identification of both autoantibody-negative and autoantibody-positive RA in the early symptomatic phase.

The set-up of the rheumatology outpatient clinic of the Leiden University Medical Centre is uniquely suited to identify patients in very early disease phases. General practitioners have been encouraged for several years to refer any patient with a suspicion of arthritis. The

start of an Early Arthritis *Recognition* Clinic (EA*R*C) in 2010, initially aimed to improve early detection of clinical arthritis, also provided an excellent opportunity to identify patients with CSA [5]. Since 2012 these patients have been included in an observational cohort and these patients were studied in this thesis.

In **Chapter 3**, we investigated the characteristics of patients with CSA at inclusion in the cohort. Subclinical inflammation of hand and foot as measured by MRI was present in 44% of the CSA patients. Subclinical MRI-inflammation was here defined as a RAMRIS MRI-inflammation score of ≥3. This cut-off was quite arbitrarily but based on MRI-findings of 19 symptom-free persons in which a score of ≥3 was rare. Furthermore, 28% of the CSA patients were positive for RA-related autoantibodies. We observed that CSA patients with MRI-inflammation were older and more frequently ACPA-positive than patients without MRI-inflammation. However, a combination of clinical and serological characteristics incompletely differentiated patients with and without MRI-inflammation. These data suggested that the information provided by MRI cannot be easily replaced by commonly used clinical and serological markers and that MRI-detected inflammation may have some diagnostic value. This was later on, in Chapter 5, further explored.

Studies on the preclinical and very early phases of RA that were performed by other groups thus far were mainly done in patients carrying autoantibodies and the very early phase of autoantibody negative RA was relatively unexplored. Therefore, in **Chapter 4**, we studied subclinical MRI-inflammation in ACPA-negative CSA patients and observed that RAMRIS MRI-inflammation scores of ACPA-negative CSA patients were significantly higher than those of 19 age-matched symptom-free controls. This suggested that ACPA-negative RA has, similar as ACPA-positive RA [6] an early phase of symptoms without clinical arthritis in which subclinical MRI-inflammation is present.

In **Chapter 5**, we studied patients with CSA and the presence of subclinical MRI-inflammation in these patients for the first time longitudinally. However, a relevant issue of the use of MRI in the early phases of RA is that MRI is a very sensitive imaging technique and it is unknown which scores should be considered as normal and which reflect pathology. Data of several MRI-studies on small numbers of symptom-free persons (including the MRI-data in symptom-free persons used in Chapter 3 and 4) showed 'MRI-abnormalities' to some extent, but were difficult to compare because different MRIs, scanning protocols and scoring methods were used [7]. Therefore, we recently performed a large-scale MRI-study in 193 symptom-free persons recruited from the general population [8]. MRI-detected inflammation was prevalent in these persons without joint symptoms as 72% had a RAMRIS MRI-inflammation score ≥1; synovitis and BME were more prevalent than tenosynovitis. MRI-inflammation was especially prevalent at higher age and at preferential locations (MCP2, MCP3, wrist and MTP1 joints). These findings suggested an influence of aging, which was observed both in these symptom-free persons as in the CSA patients studied in Chapter 3, and potentially of mechanical strains because some of the preferential locations

for MRI-inflammation in the symptom-free persons are also known as preferential location for arthritis and destruction in RA. Based on these data of MRI-findings in symptom-free persons reference values for a normal MRI were suggested. These values were specified for age, MRI-feature and anatomic location. To prevent false-positive MRIs in our CSA patients, we applied these reference values to define the presence of subclinical MRI-inflammation for our study within CSA patients.

Within our longitudinal study in patients with CSA (**Chapter 5**) we observed that 17% of all patients progressed from CSA to clinical arthritis within the first year after inclusion. Patients with subclinical MRI-inflammation had an increased risk to develop clinical arthritis as 31% of the patients with a positive MRI developed arthritis within one year. The majority did so within the first 4-5 months after inclusion, indicating that the period of CSA and subclinical inflammation is relatively short. When subclinical MRI-inflammation was absent, progression to clinical arthritis was rare (6%). In addition, we observed that tenosynovitis was more predictive than synovitis and BME as it associated independent of the other MRI-features with arthritis development. Tenosynovitis is uncommon in the general population [8], has been reported to be frequently present in early RA [9] and has been demonstrated to be present in mice in the preclinical phase before synovitis developed [10]. Together, these data may suggest that tenosynovitis may be a very early and potentially the initiating feature in arthritis development. Repeated MRI with short-time intervals during the process of arthritis development would give more insight in the timing of pathologic events occurring inside the joints. Within CSA, also ACPA-positivity associated with progression from CSA to arthritis. Both the presence of subclinical MRI-inflammation and ACPA-positivity were independently predictive for arthritis development. We used the presence and absence of MRI-inflammation and ACPA to stratify CSA patients in groups with different risks on arthritis development. Although the absolute value of MRI might be higher in ACPA-negative than in ACPA-positive CSA patients because ACPA-positive patients had already a higher prior risk of arthritis development, present data suggested that MRI is diagnostically relevant in the phase of CSA. This role is probably located in both identifying patients with an increased risk of arthritis development and ruling out imminent arthritis. The latter because the prior chance of arthritis decreased from 9% to 3% in the ACPA-negative CSA patients when the MRI was negative and from 63% to 40% in the ACPA-positive patients.

The decision on whether a patient had CSA was based on the clinical expertise of the rheumatologist as the symptoms that are characteristic for the early symptomatic phase of RA are not well-characterised. In **Chapter 6**, we studied the value of clinical expertise as selection criterion for CSA. Clinical expertise is a valuable tool in the medical and also specifically for the rheumatologic diagnostic process. For example, the clinical expertise was used in the process to develop the 2010 ACR/EULAR criteria for RA [11] and for the set-up of the French ESPOIR cohort [12]. We observed that clinical expertise is also useful for selecting arthralgia patients at risk of RA because arthralgia patients that were considered by their

rheumatologist to have CSA had an odds ratio of 55 to develop RA compared to arthralgia patients not considered to have CSA.

In **Chapter 7**, we aimed to define the clinical characteristics of patients with arthralgia who are considered at risk of RA. It can be assumed that interventions in the symptomatic phase preceding the onset of clinical arthritis may be more effective in terms of reducing the risk of disease persisting and preventing joint damage. However, studies to address this require the inclusion of homogeneous sets of patients. Therefore, with a EULAR taskforce comprising 18 rheumatologists, a methodologist, 3 health professionals, 2 patients and a research fellow we defined a set of clinical features that best characterise patients with arthralgia that are according to the clinical expert-opinion at risk of RA development. A three-phase process was used consisting of 1) identifying relevant items using a Delphi approach, 2) deriving candidate criteria by evaluating patients that were presented on paper and 3) by validating the criteria with newly referred arthralgia patients. The following set of parameters that describe arthralgia at risk of RA was defined: joint symptoms of recent-onset (duration <1 year), symptoms located in MCP joints, duration of morning stiffness 60 minutes, most severe symptoms present in the early morning, presence of a first-degree relative with RA, difficulty with making a fist and positive squeeze-test of MCP joints. In the validation phase, this combination of parameters was accurate in identifying arthralgia patients that were considered at risk of RA development as the AUC was 0.92. Test characteristics belonging to the number of positive parameters were presented and depending on the study a more sensitive or specific definition can be used.

Further perspectives on studies within CSA

In short, based on this thesis we learned that:

- The clinical expertise is useful to identify arthralgia patients who may develop RA, because patients with CSA are at increased risk of developing arthritis.
- Both ACPA-positive and ACPA-negative RA have a phase with CSA and MRI-detected subclinical inflammation.
- MRI-detected subclinical inflammation might have a diagnostic value in patients with CSA. This is true for both ACPA-positive and ACPA-negative patients but the absolute value might be higher in ACPA-negative patients.
- The developed definition of arthralgia at risk for RA which represents the consensus-based expert opinion of rheumatologist can serve as basis for future studies and trials in the CSA phase.

Our approach to study the symptomatic phase of RA without clinical arthritis is in line with the care at Dutch rheumatologic outpatient clinics. Therefore this might allow implementations of the results obtained in this thesis in Dutch rheumatologic care. However, first, replication of our findings in independent CSA populations, which do not yet exist, is needed.

To address the question whether intervention in the symptomatic phase preceding arthritis development is beneficial it is needed that patients with arthralgia with an increased risk on RA are identified. To this end, the risk factors for progression from CSA to clinical arthritis that we identified in our CSA cohort are helpful as they will contribute to accurate risk stratification within CSA. Subclinical MRI inflammation and ACPA were the most important risk factors and we performed risk stratification based on these two factors. However, our CSA population was too small to develop a full prediction model which may provide most accurate risk stratification. Such a prediction model, including all potential predictors (such as patient characteristics, symptoms, findings at physical examination, serological inflammation markers, different autoantibodies and MRI-inflammation), will reveal which factors are independently predictive for the development of arthritis and may allow to stratify the risk of arthritis/RA development more accurately. To this end, a large CSA population is needed of which part can be used for identification and the other part for validation. In addition, to perform trials in the symptomatic phase it is important to include a homogeneous group of patients. For this, the consensus expert-opinion based definition of CSA consisting of 7 clinical items is helpful. However, it is unknown how good this definition is in identifying patients who will later on progress to RA (thus the predictive accuracy of the definition). Therefore, a subsequent prospective study is needed in which patients with CSA according to the definition will be longitudinally followed on the development of RA. The diagnostic accuracy of the clinical definition alone is most likely not highly accurate because it is only based on clinical features. Presumably, combining the clinical definition with findings of additional investigations, such as results of serological tests (f.e. ACPA, RF and/or CRP) or imaging (f.e. MRI or US) will improve the diagnostic accuracy and will results in criteria for 'imminent RA'. This process is similar as the process that first led to the definition of inflammatory back pain which was subsequently integrated in the ASAS classification criteria [13,14]. Future research in which our own CSA cohort might be part of a large international prospective study with multiple cohorts will hopefully result in accurate risk stratification and can be the basis of dedicated trials.

We observed that MRI-detected subclinical inflammation might have a diagnostic value in patients with CSA and that MRI-detected tenosynovitis was most predictive for progression from CSA to clinical arthritis. This thesis comprised the first MRI-studies in patients with CSA. Further studies are needed to determine if the diagnostic value of MRI can be improved by assessing for example MRI-inflammation at specific locations or combinations of MRI-features. In addition, the value of other imaging techniques such as ultrasonography (US) in the CSA phase is not yet determined. Although MRI and US can both depict tenosynovitis, MRI has several advantages compared to US, such as that MRI is a minimal operator-dependent procedure and the presence of a validated scanning and scoring protocol for MRI [15]. If validated scanning and scoring protocol for US will become available, further studies are needed to assess the value of US in CSA.

Next to studies on predicting which CSA patients will progress to clinical arthritis, longitudinal studies within CSA patients may also provide more insight in mechanisms underlying RA development at both systemic and local levels. Biomarkers, such as gene expression profiles and autoantibody profiles can be assessed and related to MRI-detected inflammation and arthritis development. Longitudinal MRIs in individual patients will shed light on what happens inside the joints. Comparing MRIs performed in CSA and in the early clinical arthritis phase will reveal whether the extent and localisation of MRI-inflammation change during conversion from CSA to clinical arthritis.

Furthermore, additional work is needed for early identification of RA (at risk) within the primary care. General practitioners (GPs) have, as gatekeeper for access to rheumatologic care, an important role in early identification of patients with (an increased risk of) RA. GPs work in populations with different background risks and the symptoms that are characteristic for RA at risk in the GP population are unknown. Their guideline recommends that patients suspected for RA should be referred on short-term, but no specific recommendations on the symptoms and signs that should be assessed before referral are included [16]. The set-up of an EARC has improved the identification of early arthritis substantially [5], but whether knowledge on symptoms that are predictive for RA (at risk) in the GP population and the development of a referral tool would improve early identification further should be subject of subsequent studies.

Summary of research agenda:

- Replication of the findings done in our CSA cohort in independent CSA cohorts.
- To identify with high accuracy a homogenous group of CSA patients who will progress to RA. Additional risk factors for progression to RA should be identified that can contribute to dedicated risk stratification and might finally result in criteria for 'imminent RA'. The EULAR definition for CSA and European collaboration of the taskforce may be helpful to this end.
- If we can identify arthralgia patients at risk accurately, trials will reveal whether intervention can prevent onset of clinical arthritis, disease chronicity, functional disability or quality of life or whether it will reverse subclinical MRI-inflammation.
- Evaluating whether the diagnostic accuracy of MRI-detected inflammation in the CSA phase can be improved by evaluating f.e. specific locations and/or combinations of MRI-features.
- Evaluating of the value of imaging modalities such as ultrasonography and PET-CT in the CSA phase.
- Examining the sequence of pathologic events that occur in the period between onset of CSA and arthritis development both on systemic and local level in longitudinal studies.
    - Systemic level: gene expression profiles, serological inflammatory markers and epitope spreading of (different) autoantibodies.

- Local/joint level: repeated MRIs and determining the timing of the inflammatory features that are visualised (synovitis, tenosynovitis, BME, erosions).
- Development of referral tool for first line care in order to further decrease the time of referral to second line care of patients at risk of or with RA.

PART II: GENETIC FACTORS AND DISEASE OUTCOME IN RHEUMATOID ARTHRITIS

In this part, genetic risk factors for a more severe course of RA were investigated. Studying genetic variations in relation to disease outcome can increase our comprehension of disease progression, may convey novel targets for focused therapy and may improve personalised medicine. The main studied outcome was joint damage progression, one of the hallmarks of RA which can be measured objectively by scoring radiographs of hands and feet using the Sharp-van der Heijde scoring method [17]. The other studied outcome was arthritis persistence which is the other hallmark of RA and can be investigated by studying its opposite, the achievement of DMARD-free sustained remission [18].

For present studies, we selected patients fulfilling the 1987 ACR criteria for RA. In our view, these criteria for patient selection were most appropriate to perform basic research as the use of the 2010 ACR/EULAR criteria would have resulted in a more heterogeneous study population [11].

Prediction of joint damage severity on the level of the individual patients is not yet accurate, hampering individualised treatment. Matrices developed to predict rapid radiographic progression correctly classified only approximately half of all patients [19–21] and are not used in clinical practice. At the start of this thesis, several genetic risk factors had been found to be associated with joint destruction in previous studies [22–32]. When adding these genetic factors to a prediction model for radiographic progression that already included traditional factors, we observed that the predictive accuracy improved from 56% correct classifications to 62% (**Chapter 8**). In addition, genetic risk factors together explained 12-18% of the variance in radiographic progression. However, still 38% of patients were incorrectly classified by the full model and we considered the predictive performance of the derived model including genetic factors insufficient for use in clinical practice.

Replication of findings is crucial in the field of genetics. Therefore, in addition to the Leiden Early Arthritis Clinic (EAC) cohort [33], several other cohorts were used in the studies on genetic risk factors, including the Swedish Umeå [34], Spanish HCSC-RA [35], North-American Wichita [36], NDB [37] and NARAC [38], and French ESPOIR [12] cohorts. These cohorts were all smaller than the EAC cohort and comprised less radiographs over time, though could be used to replicate and substantiate observed associations. In **Chapter 9**, the initially published finding that a variant in *FOXO3A* was associated with joint damage progression in two cohorts of the UK [39] was not replicated in five other cohorts suggesting that *FOXO3A* is not a major factor regulating the severity of the course of RA.

Using candidate-gene approaches, we identified two genetic variants that were associated with joint damage progression within the ACPA-negative RA population. This is relevant because the large majority of risk variants for progression have been identified in ACPA-positive or pooled populations. First, rs9138 in *SPP1*, initially identified as susceptibility variant for RA [40] and encoding osteopontin which has a function in bone formation and remodeling was observed to associate with radiographic progression within ACPA-negative RA (**Chapter 10**). Second, in **Chapter 11**, variants that have been described to associate with radiographic progression but for which the results of different studies were incongruent were studied in six cohorts. Rs2900180 in *C5-TRAF1* significantly associated with radiographic progression; the association was confined to the ACPA-negative subgroup. The region of rs2900180 in *C5-TRAF1* was fine-mapped and another variant had a stronger association, but we could not statistically distinguish which variant was most important. The studied variants in *IL-6*, *IL-10*, and *FRCL3* were not associated and the initial findings on these variants done in studies with lower patient numbers and radiographs could be considered false-positive, underlining the relevance of replication of findings. For both rs9138 in *SPP1* and for rs2900180 in *C5-TRAF1* there was data available that the (region of the) variant is related to expression on RNA or protein level [40–42]. These studies, done on the level of genetics and expression suggested that the identified regions are relevant in pathways mediating disease progression.

The *HLA-DRB1* region is the most important genetic risk factor for both RA development and progression that is identified thus far. In particular the SE alleles, sharing a similar amino sequence at position 70-74 in the peptide-binding groove, and acting via ACPAs on disease development and outcome, are relevant [22,43,44]. However, the underlying biological pathway is not yet unravelled. Recently, a further refinement of the association of HLA and RA was proposed. Using advanced statistical methods, the strongest association with RA development was reported for HLA-DRB1 position 11 (or 13 which are in high linkage disequilibrium); this association was independent of the SE alleles [45,46]. Studying four cohorts, we observed that the amino acids Valine or Leucine at position 11 were associated with joint damage progression (**Chapter 12**). This association was independent of the presence of the SE alleles but not independent of ACPA. Future studies will reveal whether taking position 11 and 13 into account will be helpful in identifying the pathogenic antigens that result in immune activation and autoantibody production, thereby stimulating disease development and progression.

In **Chapter 13**, a candidate-gene study on arthritis persistence (the absence of achieving DMARD-free sustained remission) was performed. Genetic risk factors for joint damage progression were studied in relation to persistence and it was observed that besides the *HLA-DRB1* SE alleles, rs2104286 in *IL2RA* was associated with arthritis persistence in two cohorts. In addition, lower soluble IL2Rα (CD25) levels associated with a higher chance of remission. Intriguingly, *IL2RA* and SE are the only variants identified thus far that are

associated with RA development, joint damage progression and persistent inflammation. This underlines the relevance of these variants, but also suggested that the mechanisms driving joint damage progression and disease persistence are partially different.

In **Chapter 14**, serum level osteoprotegerin (OPG) was studied in relation to arthritis persistence. Besides the well-known role of OPG in bone metabolism, OPG also has pro-inflammatory effects and it was reported that the serum level was associated with achieving Disease Activity Score (DAS)-remission the next year [47]. Here, we replicated this latter finding. In addition, OPG level also associated with DMARD-free sustained remission. Together these data suggested that OPG levels are reflective of a process influencing the severity of inflammation both on the short and long-term.

Further perspectives on studies on risk factors for disease outcome

Including the genetic risk factors identified in this thesis, thus far, fourteen genetic variants have been identified and were replicated to associate with radiographic progression: *HLA-DRB1, CD40, IL15, DKK1, IL2RA, GRZB, IL4R, SPAG16, C5orf30, MMP9*, intergenic downstream of *ZFP36L1* and *C14orf181, OPG, SPP1* and *C5-TRAF1*. We observed that these variants together explained approximately 20% of the variance in radiographic progression. This cannot be directly compared to the estimation that 45-58% of the severity of joint damage is heritable which was estimated in Icelandic RA patients [48], but it suggests that part of the heritability is still missing. In line with this, we observed that radiographic progression could not be accurately predicted using all known, both traditional and genetic, risk factors. This 'missing heritability' might be explained by not yet identified common genetic variants that associate with joint damage, rare variants with large effects on joint damage or by gene-gene or gene-environment interactions. To this end, radiographic data of several cohorts should be combined to enable large studies.

The other long-term outcome that was studied was arthritis persistence (the absence of achieving DMARD-free sustained remission). It is likely that achieving DMARD-free sustained remission will become a preferred treatment goal in the future, but only few risk factors for arthritis persistence are known thus far. We performed a candidate-gene study and hypothesised that genetic risk factors for joint damage might also be risk factors for arthritis persistence. This approach sounds reasonable as both outcomes are a reflection of the long-term disease course. However, in fact there is no clear evidence that underlying processes of joint damage and arthritis persistence are overlapping. In addition, it is unclear whether the patients with severe joint damage are similar to the patients with persistent arthritis. Ideally, we had performed a hypothesis-free genome-wide association study (GWAS) or had analysed the whole Immunochip [49] in relation to arthritis persistence. Unfortunately, this was hampered by the low frequency of DMARD-free sustained remission and the absence of multiple cohorts with data on this disease outcome. Collection of data on this outcome in multiple cohorts would allow such large genetic studies.

Thus far, multiple genetic risk factors have been identified for RA development or disease progression within RA. Previously, it was observed that the genetic variants that are associated with susceptibility to RA and joint damage progression of RA are largely different [30]. In this thesis, we studied the genetic risk factors for joint damage, which are mainly located in genes involved in inflammation, immunity or bone/cartilage metabolism, in relation to arthritis persistence and observed that also these were largely non-overlapping. The *HLA-DRB1* alleles and a variant in *IL2RA*, both located in genes involved in immunity/inflammation, were the only variants that associated with both joint damage progression and arthritis persistence. Variants in genes involved in bone/cartilage metabolism associated with joint damage, but not with arthritis persistence. Overall, these data suggest that the processes driving the development of RA, progression of joint damage within RA and persistence of arthritis within RA are largely different. However, further studies are needed to unravel these pathways and to give more insight in whether the identified variants are causal and how these variants are involved in disease development and disease progression. For this, large fine-mapping studies and functional studies are needed, respectively to identify all variants that are linked to the variants with the strongest association and to determine the potential functional consequences of these variants. Recent advances in technology and bioinformatics may be helpful to this end [50].

In addition, we found 2 serological biomarkers that were associated with arthritis persistence, i.e. high soluble IL2Rα and OPG levels. These findings might give additional clues for targeted intervention. Interestingly, lowering soluble IL2Rα (CD25) levels with anti-CD25 (daclizumab) have been shown to be effective in multiple sclerosis [51]. In addition, upregulation of regulatory T-cells with low-dose IL2 was beneficial in type 1 diabetes [52]. Further research on longitudinal measured biomarker levels would reveal the relevance of specific serological biomarkers for arthritis persistence and their potential role in guiding treatment decisions.

PART III: OTHER OUTCOMES IN RHEUMATOID ARTHRITIS

Due to improved treatment strategies, severe joint damage is less prevalent nowadays and therefore, other outcome measures will become more important. A good candidate would be arthritis persistence and its opposite achieving DMARD-free sustained remission, which is the closest proxy of cure of RA and can be assessed rather objectively [18]. This outcome was used in Chapter 13 and 14. In **Chapter 15**, the occurrence and relevance for patients of achieving DMARD-free sustained remission was studied. We observed that with nowadays treatment strategies the chance to achieve this favourable outcome is increased. It was observed that also from patient perspective achieving DMARD-free sustained remission is an outcome to pursue as this status reflected resolution of symptoms and disability. This underlines the relevance of this outcome.

Patient-reported outcomes such as fatigue, functional ability and work ability are

also important [53] outcome measures in RA. Fatigue is a frequently reported symptom in RA, associated with functional disability and considered one of the most important outcomes by patients. The causation of fatigue in RA is thought to be multidimensional [54] and the contribution of inflammation in unclear. We studied the long-term course of fatigue (**Chapter 16**) and observed that fatigue is a persistent problem in RA. In addition, the extent of inflammation over time significantly associated with the severity of fatigue though the effect sizes were small, indicating that non-inflammatory pathways should be considered important as well. Interestingly, improved treatment strategies that have resulted in less inflammation and improved objective outcomes of RA have not resulted in less severe fatigue. Therefore, fatigue in RA remains an 'unmet need'.

FINAL CONCLUSIONS

The field of RA is moving into identification of patients as early as possible and the ultimate aim is to prevent RA becoming a chronic disease. To this end, the studies on the phase of Clinically Suspect Arthralgia (CSA), described in Part I of this thesis provided relevant insights. Patients with arthralgia that were considered by the rheumatologist to have an increased risk to progress to RA (CSA) had indeed an increased risk of RA. In addition, subclinical MRI-inflammation preceded clinical arthritis with a few months. Future research will shed more light on processes underlying progression from CSA to RA and effectiveness of treatment initiation in the CSA phase.

The severity of the course of RA is variable between patients and this cannot be yet accurately predicted. The studies in Part II and III contributed to the understanding of these differences in severity. Three genetic risk factors for more severe joint damage progression (two non-HLA and one HLA variation) and one for arthritis persistence were identified. Further research on functional implications of the identified variants and whether they might be useful as biomarkers to guide treatment decisions is needed.

DMARD-free sustained remission, the opposite of arthritis persistence, will probably become an increasingly important outcome in RA as it approximates cure of RA, is relevant from patient perspective and is increasingly achievable nowadays although the majority of patients is not yet able to achieve this outcome. Future studies will reveal whether this beneficial outcome can be achieved more frequently when treatment is initiated in the phase of CSA.

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Nederlandse samenvatting

18

Reumatoïde artritis (RA) is een chronische ontstekingsziekte van met name de kleine gewrichten van de handen en voeten. Het is een veelvoorkomende ziekte, wereldwijd bij 0.5-1% van de bevolking, en treft vooral vrouwen van middelbare leeftijd. De gewrichtsontstekingen (artritis) die kenmerkend zijn voor RA gaan gepaard met pijn, zwelling en stijfheid en kunnen, als de ziekte niet goed behandeld wordt, leiden tot e destructie. RA is een systeemziekte wat inhoudt dat niet alleen de gewrichten, maar ook andere organen zoals de huid, ogen, hart en longen betrokken kunnen zijn bij de ziekte. Daarbij zijn systemische symptomen zoals vermoeidheid vaak aanwezig en is er bij RA een verhoogd risico op hart en vaatziekten.

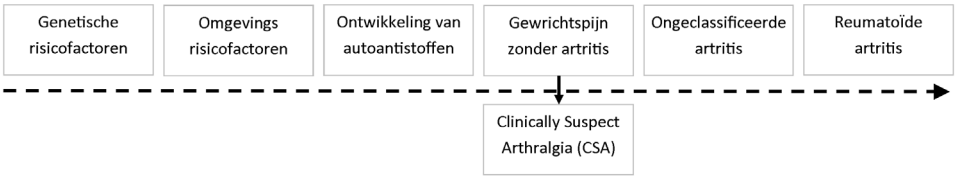
De oorzaken voor het ontstaan van RA zijn nog grotendeels onduidelijk, maar het wordt beschouwd als een auto-immuunziekte, omdat een groot deel van de patiënten autoantistoffen heeft. De autoantistoffen RF (reumafactor) en/of ACPA (anti-citrullinated peptide antibodies) komen bij ongeveer twee derde van de patiënten voor en vooral ACPA is kenmerkend voor RA. Waarom deze antistoffen aanwezig zijn en wat ze precies doen, is onbekend. Wel worden ACPA-positieve en ACPA-negatieve RA als twee subentiteiten van RA beschouwd met een verschillende genetische achtergrond en verschillend ziekteverloop, waarbij ACPA-positieve RA ernstiger is. Meer dan 100 genetische varianten zijn tot nu toe ontdekt die leiden tot een verhoogd risico op het ontwikkelen van RA. Dit geeft het belang van erfelijke factoren aan voor het ontstaan van RA. De meeste genetische risicofactoren zijn ontdekt voor ACPA-positieve RA waarvan de belangrijkste een groep HLA-genen zijn (de zogenaamde shared epitope allelen). Ook omgevingsfactoren spelen waarschijnlijk een rol bij het ontstaan van RA. Mensen die roken hebben een verhoogd risico op het krijgen van RA.

De behandeling van RA is de laatste twee decennia sterk verbeterd waardoor ernstige gewrichtsschade minder vaak voorkomt en het steeds vaker mogelijk wordt om remissie te bereiken en te behouden. Hieraan hebben zowel de ontwikkeling van nieuwe effectieve medicatie (disease-modifying antirheumatic drugs (DMARDs) en biologicals) als de verschuiving van een afwachtend beleid naar een beleid van direct ingrijpen als de ziekte zich presenteert bijgedragen. De bevinding dat het vroeg starten van de behandeling gunstig is voor het ziekteverloop heeft ertoe geleid dat de nadruk tegenwoordig ligt op het zo vroeg mogelijk herkennen van RA.

DE FASE MET CLINICALLY SUSPECT ARTHRALGIA

Onderzoek heeft uitgewezen dat de ontwikkeling van RA een proces van jaren kan zijn. Zo kunnen de autoantistoffen al lang voordat de ziekte zich manifesteert aanwezig zijn. Ook kan aan het ontstaan van een klinisch detecteerbare artritis (een gezwollen gewricht bij lichamelijk onderzoek) een periode voorafgaan waarin gewrichtsklachten aanwezig zijn, maar er nog geen artritis is (het gewricht is niet gezwollen) (Figuur). Het onderzoeken van deze vroegste fasen, voordat er artritis is, kan leiden tot een beter begrip van het ontstaan van RA en biedt een mogelijkheid om patiënten met RA nog vroeger te herkennen dan we nu doen. Het eerste deel van dit proefschrift richtte zich op de fase met gewrichtspijn zonder artritis.

Figuur. De fasen van RA-ontwikkeling



Hoewel het duidelijk is dat een periode met gewrichtspijn zonder artritis voorafgaat aan het ontstaan van een klinische artritis is het onbekend welk type klachten kenmerkend zijn voor deze zeer vroege fase. In dit proefschrift onderzochten wij deze fase door patiënten met Clinically Suspect Arthralgia (CSA), klinisch verdachte gewrichtspijn (artralgie), te onderzoeken (Figuur). Dit is pijn van de kleine gewrichten die de reumatoloog vanwege het karakter van de symptomen verdacht vindt om zich ooit tot RA te ontwikkelen. Het besluit of een patiënt CSA heeft, is dus vooral gebaseerd op de klinische expertise, of het 'onderbuikgevoel' van de reumatoloog dat de symptomen een uiting van beginnende RA kunnen zijn. Sinds 2012 worden deze patiënten in een cohortonderzoek in het LUMC onderzocht en worden ze in de tijd gevolgd op het ontwikkelen van een klinische artritis/RA. Van de patiënten met CSA wordt een MRI maakt van de meest pijnlijke hand en voet om uit te zoeken of er ontsteking zichtbaar is op de MRI die (nog) niet met het lichamelijk onderzoek gedetecteerd kan worden; dit noemen we subklinische MRI-ontsteking. MRI is een zeer sensitieve methode om ontsteking van het gewrichtskapsel, bot en pezen weer te geven. Met behulp van een score worden de MRI's beoordeeld op de aanwezigheid van ontsteking. De studies die zijn beschreven in dit proefschrift zijn voor een groot deel uitgevoerd in het Leidse CSA-cohort.

In de eerste hoofdstukken van dit proefschrift werd het CSA-cohort geïntroduceerd en werden de baselinebevindingen van patiënten met CSA beschreven. De belangrijkste redenen die de reumatologen aangaven waarom ze de gewrichtspijn klinisch verdacht vonden om ooit RA te worden, was gewrichtspijn die in de ochtend het ergst was en minder werd gedurende de dag, de aanwezigheid van ochtendstijfheid van minimaal één uur en het voorkomen van RA in de familie van de patiënt. Subklinische MRI-ontsteking was aanwezig bij 44% van de patiënten met CSA. Patiënten met en zonder MRI-ontsteking hadden vergelijkbare symptomen en karakteristieken en deze patiënten waren dus niet goed op basis van hun symptomen en karakteristieken van elkaar te onderscheiden. MRI gaf dus aanvullende informatie over een patiënt met CSA.

Omdat MRI zeer sensitief is, was het belangrijk om te weten welke MRI-score als abnormaal beschouwd kon worden. Daarom hebben we een studie uitgevoerd waarin MRI's van de hand en voet van 193 personen zonder gewrichtsklachten zijn gemaakt. Dit liet zien dat MRI-ontsteking vaak voorkwam bij symptoomvrije personen en dus niet alleen bij patiënten met RA/CSA; 72% van de personen zonder klachten had een MRI-score van minimaal 1

voor ontsteking. MRI-ontsteking kwam vooral voor bij oudere personen en in bepaalde gewrichten (zoals MCP2 en MCP3 (wijs- en middelvinger) en MTP1 (grote teen)). Met behulp van deze data hebben we referentiewaarden opgesteld voor een 'normale' MRI. Deze referentiewaarden waren gespecificeerd voor leeftijd en type en locatie van de ontsteking. In vervolgonderzoek bij CSA-patiënten hebben we deze referentiewaarden gebruikt om een 'positieve' MRI voor ontsteking te definiëren. Hierdoor voorkwamen we dat de MRI's van CSA-patiënten onterecht als positief werden beschouwd.

De eerste longitudinale studie van patiënten met CSA toonde aan dat 17% van de patiënten in het eerste jaar een klinische artritis had ontwikkeld. Deze overgang van CSA naar artritis gebeurde vooral in de eerste paar maanden. Het gevoel van de reumatoloog dat een patiënt een verhoogd risico had om RA te krijgen, was dus voor een deel van de patiënten terecht. Patiënten die een positieve MRI hadden, hadden een extra verhoogd risico om artritis te krijgen; 31% van hen ontwikkelde artritis in het eerste jaar. Ook patiënten die ACPA-positief waren, hadden een verhoogd risico op artritis en zowel binnen de ACPA-positieve als de ACPA-negatieve CSA-patiënten voorspelde de aanwezigheid van een positieve MRI het ontstaan van artritis. Dit gaf aan dat MRI een toegevoegde waarde had in het identificeren van degenen die artritis gingen krijgen. Hiernaast had MRI ook een rol in het identificeren van de CSA-patiënten die juist een kleine kans hadden om artritis te ontwikkelen. De kans om artritis te ontwikkelen was namelijk slechts 6% als de MRI negatief was voor ontsteking. Deze studie toonde dus aan dat MRI waardevol was om binnen de totale groep CSA-patiënten onderscheid te maken tussen patiënten met een hogere kans en met een lage kans op toekomstige RA.

De laatste hoofdstukken van het eerste deel gingen over de definitie van CSA. Het besluit of een patiënt CSA had, was gebaseerd op de klinische expertise van de reumatoloog. De klinische expertise wordt vaak gebruikt in de geneeskunde, bijvoorbeeld door huisartsen die het klinische beeld 'pluis' of 'niet pluis' vinden. Ook voor het besluit of er CSA was, bleek de klinische expertise een nuttig middel. Patiënten met gewrichtspijn die door de reumatoloog werden beschouwd als CSA hadden ongeveer 55 keer meer kans om RA te krijgen dan gewrichtspijn patiënten zonder CSA.

Behalve dat gewrichtspijn klinisch verdacht moest zijn om RA te worden, hadden we geen verdere criteria gedefinieerd waaraan CSA moest voldoen. Echter, als we interventiestudies in de CSA-fase zouden willen uitvoeren om te onderzoeken of het starten van behandeling in de CSA-fase gunstig is, is het belangrijk dat een homogene patiëntengroep met CSA geselecteerd kan worden. Hiervoor waren duidelijke criteria nodig. Daarom hebben we met een groep van 18 reumatologen uit heel Europa een definitie voor CSA ontwikkeld. Deze definitie, bestaande uit 7 klinische parameters, representeert de gedeelde opvattingen van wat de reumatologen beschouwden als gewrichtspijn die verdacht was om RA te worden, dus CSA. Of deze definitie daadwerkelijk voorspellend is voor het ontwikkelen van RA moet

nog onderzocht worden.

HET VERLOOP VAN REUMATOIDE ARTRITIS - GENETISCHE RISICOFACTOREN

In het tweede en derde deel van dit proefschrift werden patiënten met RA onderzocht. In tegenstelling tot de patiënten met CSA in het eerste deel van dit proefschrift, hadden deze patiënten dus al de diagnose RA.

Het ziekteverloop van RA kan veel verschillen tussen patiënten. Sommige patiënten hebben een invaliderend en persisterend verloop met veel gewrichtsschade terwijl bij anderen de ziekte milder verloopt. Waarom de ene patiënt een ernstiger ziekteverloop heeft dan de andere patiënt is onbekend; we kunnen dit ook niet goed voorspellen. Dus om beter inzicht te krijgen in mechanismen die verantwoordelijk zijn voor ziekteprogressie en om behandeling te individualiseren is het belangrijk om risicofactoren te identificeren voor een ernstig ziekteverloop. Het tweede deel van dit proefschrift richtte zich op genetische risicofactoren voor een ernstig verloop van RA.

De onderzoeken in dit deel van het proefschrift zijn grotendeels uitgevoerd in het Leidse Early Arthritis Clinic (EAC) cohort. De EAC is een cohortonderzoek dat in 1993 is gestart en waarin sindsdien alle patiënten die zich op de polikliniek reumatologie van het LUMC presenteren met een nieuwe artritis geïncludeerd worden. Echter, aangezien binnen de wetenschap, en speciaal binnen het veld van de genetica, replicatie van bevindingen essentieel is, bevatten veel studies in dit proefschrift resultaten van meerdere cohorten met RA-patiënten, afkomstig uit Frankrijk, Zweden, Spanje en de Verenigde Staten.

De ernst van RA kan met verschillende maten gemeten worden. De belangrijkste langetermijnuitkomst is de ernst van schade aan de hand- en voetgewrichten die bepaald kan worden op radiologische foto's. Deze uitkomstmaat heeft enkele voordelen: (1) gewrichtsschade geeft de ziektegeschiedenis weer, aangezien er wordt aangenomen dat schade zich niet of nauwelijks herstelt, (2) gewrichtsschade is sterk geassocieerd met andere uitkomstmaten zoals functionaliteit en (3) er zijn net als voor MRI gevalideerde scoringsmethoden beschikbaar om gewrichtsschade te scoren. De meest gebruikte scoringsmethode is de Sharp-van der Heijde score waarbij erosies en gewrichtsspleetvernauwing in de handen en voeten worden beoordeeld.

Er zijn al verscheidene risicofactoren bekend voor de ernst van gewrichtsschade. De belangrijkste is de aanwezigheid van autoantistoffen. Onderzoek heeft aangetoond dat erfelijkheid ook een belangrijke rol speelt in het ontstaan van schade: 45-58% in de variantie van ernst in schade is erfelijk. Hoewel er al meerdere genetische risicofactoren voor schade geïdentificeerd en gerepliceerd zijn, is een groot deel van het genetische effect nog onverklaard.

Er zijn verscheidene voorspelmodellen voor de ernst van gewichtsschade ontwikkeld. Deze modellen, gebaseerd op kenmerken zoals aantal gezwollen gewrichten, antistoffen

en ontstekingswaarden, voorspelden echter de ernst van de schade in slechts 50% van de patiënten correct. Ze worden daarom niet in de klinische praktijk gebruikt. Wij onderzochten of de voorspelling beter zou worden als ook rekening gehouden werd met genetische risicofactoren voor gewrichtsschade. Ons model met alleen klinische factoren classificeerde 56% van de patiënten correct en een model met ook genetische factoren 62%. Hoewel dit een verbetering in voorspellend vermogen was, werd nog steeds bij 38% van de patiënten de ernst van de gewrichtsschade incorrect voorspeld. Daarom beoordeelden wij het model dat rekening hield met genetische factoren nog steeds als onvoldoende.

Dit proefschrift beschreef enkele kandidaat-gen studies naar genetische risicofactoren voor de ernst van het verloop van RA. Een kandidaat-gen studie betekent dat je hypothese gedreven een bepaald (deel van een) gen gaat onderzoeken. We identificeerden twee genetische risicofactoren voor gewrichtsschade in ACPA-negatieve RA-patiënten; dit ging om variaties in de genen *SPP1* en *C5-TRAF1*. Deze bevindingen zijn relevant, aangezien de meeste genetische risicofactoren voor schade geïdentificeerd zijn in ACPA-positieve of een gepoolde ACPA-positieve en ACPA-negatieve populatie. Bovendien waren beide variaties geassocieerd met expressie van RNA of eiwit. Dit versterkte het bewijs dat de geïdentificeerde varianten (of de regio waarin ze liggen) mogelijk relevant zijn voor processen die betrokken zijn bij ziekteprogressie. Verder onderzoek hiernaar is nodig.

De belangrijkste genetische risicofactoren voor gewrichtsschade zijn, net als voor RA ontwikkeling, de *HLA-DRB1* shared epitope (SE) allelen, die coderen voor dezelfde aminozuurvolgorde op positie 70-74 van het *HLA-DRB1* molecuul. Deze associatie wordt gemedieerd door ACPA wat inhoudt dat de SE-allelen leiden tot ACPA wat leidt tot de ontwikkeling van RA en ernstige schade als er RA is. Recent was aangetoond dat variaties in positie 11 van het *HLA-DRB1* gen sterker dan en onafhankelijk van de SE-allelen associeerden met de ontwikkeling van RA. Wij toonden aan dat positie 11 op *HLA-DRB1* ook associeerde met gewrichtsschade en dat dit tevens onafhankelijk was van de aanwezigheid van SE-allelen. De associatie was echter net als de associatie van SE niet onafhankelijk van ACPA.

Een andere belangrijke langetermijnuitkomst is het bereiken van DMARD-vrije remissie. Dit houdt in dat na het staken van medicatie er bij het lichamelijk onderzoek geen artritis meer is en is dus het tegenovergestelde van persisterende ziekte. RA werd altijd gezien als een chronische ziekte die niet te genezen was. Echter, het bereiken van DMARD-vrije remissie kan beschouwd worden als een sterke benadering van genezing en wij lieten zien dat deze uitkomst met de huidige behandelstrategieën steeds vaker behaald kon worden. Ook vanuit het perspectief van de patiënt was dit een na te streven uitkomst, omdat patiënten in remissie geen symptomen en functiebeperking meer ervoeren. Met het afnemen van ernstige gewrichtsschade zal het bereiken van DMARD-vrije ziekte waarschijnlijk een steeds belangrijkere uitkomst worden.

Om meer inzicht te krijgen in de vraag waarom de ene patiënt een persisterende

chronische ziekte heeft en de andere patiënt in remissie gaat, hebben we een kandidaat-gen studie gedaan naar DMARD-vrije remissie. Een variant in *IL2RA* bleek geassocieerd met deze uitkomst. Ook het level IL2Rα wat meetbaar is in het bloed was geassocieerd met remissie. Tot nu toe is *IL2RA* naast *HLA-DRB1* de enige genetische risicofactor voor het ontwikkelen van RA, de ernst van gewrichtsschade en het bereiken van remissie. Gezien de potentiële relevantie van *IL2RA* voor RA, zou het interessant zijn om te onderzoeken of medicatie die aangrijpt in het IL2 mechanisme en effectief is gebleken in andere auto-immuunziekten effectief is voor de behandeling van RA.

CONCLUSIES

Vroege herkenning van patiënten met RA is belangrijk. De studies over de fase met Clinically Suspect Arthralgia (CSA) die beschreven zijn in dit proefschrift dragen bij aan inzicht in de vroegste fasen van RA-ontwikkeling. Patiënten met klinisch verdachte gewrichtspijn (CSA) hebbenn een verhoogd risico op het ontwikkelen van artritis. Subklinische MRI-ontsteking gaat een aantal maanden aan de ontwikkeling van artritis vooraf. Verder onderzoek zal meer inzicht geven in de processen die een rol spelen bij progressie van CSA naar RA, de toepasbaarheid van MRI in de klinische praktijk en de effectiviteit van behandeling in de CSA-fase.

Het ziekteverloop van patiënten met RA kan erg verschillen en we weten niet goed welke factoren hieraan bijdragen. In het tweede deel van dit proefschrift identificeerden we drie genetische risicofactoren voor gewrichtsschade en één voor persisterende ziekte. Aangezien gewrichtsschade tegenwoordig minder vaak voorkomt door de huidige behandelstrategieën en het bereiken van DMARD-vrije remissie een haalbare uitkomst is geworden, zal deze uitkomst steeds belangrijker worden in RA.

Curriculum Vitae

Hanna van Steenbergen werd op 10 mei 1988 geboren in Delft. In 2006 behaalde zij cum laude haar gymnasium diploma aan het Stanislascollege in Delft. Hetzelfde jaar begon ze haar studie geneeskunde in Leiden. Tijdens de laatste fase van haar studie deed zij op de afdeling Trombose & Hemostase van het LUMC onderzoek naar postpartum bloedingen bij patiënten met van Willebrand ziekte en draagsters van hemofilie (onder begeleiding van Prof. dr. HCJ Eikenboom). Ze rondde haar studie af me een keuze-coschap op de afdeling reumatologie van het HAGA-ziekenhuis (Den Haag) en een semi-arts stage op de afdeling interne geneeskunde van het Bronovo ziekenhuis (Den Haag). In oktober 2012 werd het artsexamen cum laude behaald.

Hierna startte zij met haar promotie onderzoek op de afdeling reumatologie van het Leids Universitair Medisch Centrum onder begeleiding van Prof. dr. AHM van der Helm-van Mil en Prof. dr. TWJ Huizinga. Als eerste onderzoeker was zij werkzaam op het Leidse Clinically Suspect Arthralgia (CSA) cohort. Ook deed zij onderzoek naar genetische factoren die geassocieerd zijn met een ernstig verloop van reumatoïde artritis. In het laatste jaar van haar promotietraject was zij als research fellow betrokken bij een EULAR project om een definitie voor Clinically Suspect Arthralgia te ontwikkelen.

In december 2015 is zij gestart met haar opleiding tot reumatoloog in het LUMC. De interne vooropleiding volgt zij momenteel in het Bronovo ziekenhuis in Den Haag (opleider Dr. YWJ Sijpkens).

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