Comprehending the symptomatic phase preceding rheumatoid arthritis: Clinically suspect arthralgia

Brinck, R.M. ten

Comprehending the symptomatic phase preceding Rheumatoid Arthritis: Clinically Suspect Arthralgia

Robin Marco ten Brinck

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Chapter 1

Introduction

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a disease characterized by chronic inflammatory joint disease with persistent synovitis and systemic inflammation.[1] It can cause permanent deformities by destruction of cartilage and bone.[1] If left untreated, RA will often lead to loss of physical functioning, the inability to carry out activities of daily living and a reduction of work ability.

RA is one of the most common inflammatory arthritides, and it has been estimated that RA affects about 1% of the population.[2] Occurrence of RA is more frequent on the Northern hemisphere and in urban areas.[2,3] Data collected by Dutch general practitioners determined that the prevalence of RA in the Netherlands in 2017 was approximately 1.5%.[4] RA predominantly occurs in females (female to male ratio: 3:1) and the prevalence rises with age, although it can arise at all ages.[5] Several risk factors for RA have been assessed in recent years.[6] These include genetic risk factors[7] as well as environmental risk factors[8], of which repeated activation of innate immunity and exposure to tobacco smoke are, putatively, the most important.[9]

Evidence suggests that RA arises from a combination of multiple hits, in which environmental, lifestyle, and stochastic insults occurring in a genetically predisposed, epigenetically modified individual leads to a breakdown of immunological tolerance.[1] This breach leads to a crucial transition towards the chronic (non-resolving) autoimmune synovitis delineating RA.[1]

The typical presentation of 'classic' RA is a middle-aged woman with subacute smouldering polyarticular, symmetric arthralgia and swelling of small joints in the hands and feet. Quintessentially, the metacarpophalangeal (MCP), proximal interphalangeal (PIP), wrist and metatarsophalangeal (MTP) joints are involved. Other characteristic

signs and symptoms include morning stiffness, fatigue and weight loss. Furthermore, extraarticular manifestations may exist, such as skin abnormalities (rheumatoid nodules), pulmonary or cardiac involvement, decreased psychological well-being and vasculitis.[10,11] Systemic comorbidities (e.g. cardiovascular) can be present.[10,12] Physical examination should always assess joints for the presence and distribution of tender and swollen joints. Laboratory testing may yield elevated levels of autoantibodies: anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). Furthermore, acute phase reactants such as the erythrocyte sedimentation rate and C-reactive protein levels are typically elevated in RA.

Identification and development of RA

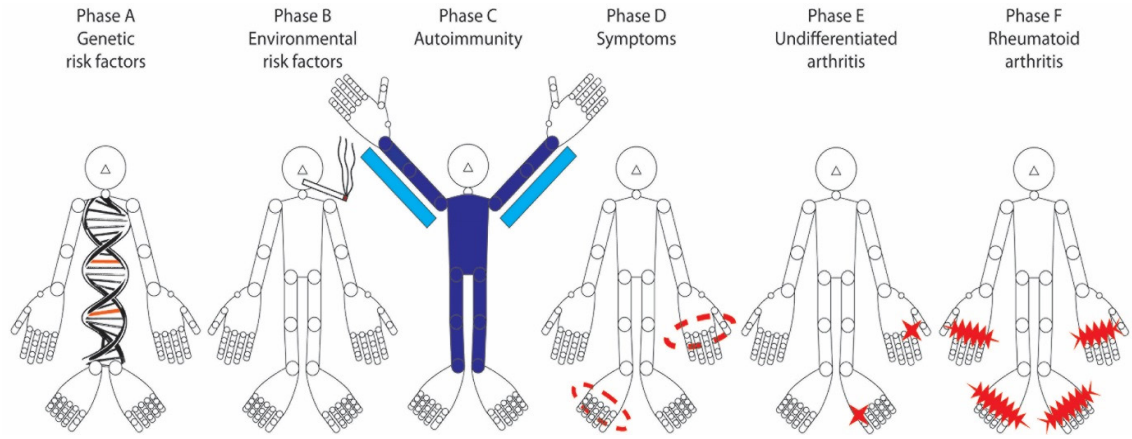
Identifying patients in the early symptomatic phase of RA may present a challenge to clinicians. Especially in primary care, the high incidence of consultations for various common musculoskeletal symptoms is mirrored by a low incidence of actual early RA.[4] The consultation prevalence of any musculoskeletal symptom in primary care approximates 2405 per 10,000 per year [4,13], making it the most common organ system consulted for at GP practices.[4,13,14] Despite this, the majority of all arthralgia patients will never develop RA, with the average full-time GP diagnosing only one new patient with RA each year.[15] Nevertheless, general practitioners (GPs) in the Netherlands generally do well in titrating those patients with RA for referral for secondary care. From that point onwards, diagnostic workup should be executed by rheumatologists.

The existence of a phase of preclinical disease in RA was evinced by observations from studies in blood donors from whom multiple blood samples were available years before their arthritis became clinically apparent.[16–18] These observations, in addition to evidence of epitope spreading[19,20] and elevated markers of systemic inflammation[21,22] suggest a maturation of the inflammatory response during the years before a patient will present with RA.

During these years, patients can already advance to a symptomatic phase in which RA could, ideally, be identified.[23]

One might wonder why early identification of RA is such a key issue from a clinician's point of view. Early identification allows the initiation of early treatment. The 2016 update of EULAR recommendations for the management of RA recommends starting therapy with Disease-modifying antirheumatic drugs (DMARDs) as soon as the diagnosis of RA is made.[24] Underlying the significance of early treatment initiation is the possibility of a 'window of opportunity'.[25] This 'window' presumes the existence of a confined period in which RA is most susceptible to the disease-modifying effects of treatment. It is postulated that the disease is more prone to respond to DMARDs because underlying disease processes have not yet fully matured.[26] The main therapeutic target is indeed reversal of the inflammatory response; if inflammation diminishes rapidly, structural damage is prevented and physical functioning can be amended without sequelae. Delay of treatment initiation was shown to be associated with worse disease outcomes, including more severe structural damage and a lower likelihood of achieving remission.[25,27,28]

A study group from the European League Against Rheumatism (EULAR) defined recommendations for nomenclature to be used to describe the aforementioned (pathophysiological) phases in the development of RA.[5] Phase A comprises genetic risk factors, whereas phase B specifies environmental risk factors. Next, phase C is characterized by autoimmunity associated with RA. Phase D is the first phase in which symptoms are present: patients experience arthralgia but no synovitis. Unclassified arthritis (and clinical synovitis) is present in phase E and, finally, there is phase F in which patients can be classified as having RA. These phases are depicted in Figure 1.



Legend: Image from: van Steenbergen et al, *Arthritis Rheum*. 2013.[23]

The paramount challenge from a clinician's standpoint is to identify patients with RA in an early phase of clinical arthritis. A key question is whether patients with RA can be identified in an even earlier phase, before clinical arthritis occurs. The presumption of the latter is that intervention in the symptomatic preclinical phase of RA would be even more effective than during the phase of early clinical arthritis.

Strategies to identify RA in the phase preceding clinically apparent arthritis

One strategy for the identification of persons at risk for RA is laboratory testing of autoantibodies in at-risk populations (e.g. in persons with a positive family history, or in non-selected populations such as persons visiting health fairs) in which rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) will be determined. If these antibodies are present, persons are considered to be at risk for the development of RA.[16,29] The additional value of other predictors (such as imaging abnormalities) in these populations has also been studied.[30,31] A potential advantage of this approach is that it can be applied by primary

care physicians as well, and rheumatologic evaluation is not required before performing autoantibody testing. Disadvantageous of this approach is that the prior risk is lower if the clinical expertise is not used as selection criterion. In addition, this strategy excludes early identification of RA patients in which these factors are not present.

Another strategy to identify patients at risk for developing RA is to start with clinical evaluation and use of the clinical expertise of rheumatologists. Throughout this thesis, this method is used for the identification of patients at risk for RA. Using pattern recognition in signs and symptoms reported by patients, rheumatologists can identify patients with Clinically Suspect Arthralgia (CSA). This approach is likely applied by all rheumatologists – whether knowingly or unknowingly – in their daily practise when evaluating patients presenting with (recent-onset) arthralgia. It is noteworthy that patients that are identified with CSA by their rheumatologists comprise a small group of all patients presenting with arthralgia to secondary care (<6%).[32] Despite encompassing a small group, the odds ratio of CSA patients to be subsequently diagnosed with RA was high: 55.[32] However, the CSA-approach is only feasible in secondary care. It is independent of autoantibodies and therefore includes autoantibody-positive and -negative patients. Thereafter, the discriminative value of different biomarkers such as autoantibodies and imaging can be studied. A disadvantage of this approach is that it is to some extent subjective, as CSA is demarcated by the clinical expertise of rheumatologists.

To increase homogeneity, a EULAR taskforce recently defined a set of clinical characteristics for arthralgia suspicious for progression to RA.[33] It has been shown that this definition indeed increased the inclusion of homogeneous sets of patients with an increased rate of RA development: patients with a clinical suspicion and a positive EULAR definition had a two times increased hazard to progress to RA compared to patients with a clinical suspicion but a negative definition.[34] The parameters defined

are: 1. symptom duration <1 year, 2. symptoms localized in the MCP joints, 3. morning stiffness lasting ≥60 min, 4. most severe symptoms experienced in the early morning, 5. having a first-degree relative with RA, 6. difficulty with making a fist, and 7. a positive squeeze test of the MCP joints (Table 1). If a sensitive definition is preferred, the suggested cut off is 3 parameters present.[33] This definition will be used in several of the chapters included in this thesis.

Table 1. EULAR-defined characteristics describing arthralgia at risk for RA

History taking:

- Joint symptoms of recent onset (duration <1 year)
- Symptoms located in MCP joints
- Duration of morning stiffness ≥60 min
- 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

The Clinically Suspect Arthralgia cohort

CSA patients are included in a longitudinal cohort in Leiden since 2012; this cohort formed the basis for the studies presented in this thesis. The Leiden Clinically Suspect Arthralgia cohort is an inception cohort at the rheumatology outpatient clinic of the Leiden University Medical Centre, the Netherlands. Per definition, CSA patients had recent-onset (<1 year) arthralgia of hand (MCP, PIP, wrist) or feet (MTP) joints, and they were considered at risk for RA. Patients were indicated as having CSA based on the first clinical presentation. Patients did not have CSA – and would therefore not be included in the cohort – if clinical arthritis was already present at baseline physical examination, or if another explanation for the pain was more likely. Examples of this includes presence of tenderpoints (indicative of fibromyalgia), or presence of Heberden

or Bouchard nodules (indicating osteoarthritis).

As GPs in the Leiden region are discouraged from performing autoantibody testing before referral to secondary care, ACPA- and RF-status were generally unknown at baseline presentation. Hence, autoantibody-positive patients could also be included in the cohort as long as the rheumatologists considered the pattern of CSA present. This is a clear distinction with the approach in which only autoantibody-positive at-risk patients are studied. Furthermore, MR-imaging in the CSA cohort was performed within 2 weeks after inclusion and therefore the status regarding the presence or absence of baseline imaging abnormalities was also unknown at inclusion in the cohort.

At baseline inclusion in the CSA cohort, questionnaires were completed, physical examination performed, blood obtained and imaging (X-rays and MRI) performed. MR-imaging is performed on the MCP2–5, wrist and MTP1–5 joints of the most painful side, or the dominant side case of equally severe symptoms at both sides. The joints were scanned with an 1.5 Tesla extremity MRI-scanner using contrast-enhancement with gadolinium and scored according to the RA MRI scoring system (RAMRIS) protocol.[35,36]

Patients were prospectively followed with scheduled visits at 4, 12 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. This provided early access to rheumatology care if patients developed clinically evident synovitis and thus inflammatory arthritis was identified at the first possible opportunity. Follow-up in the CSA cohort ended if either clinical arthritis was identified or at the final visit at 24 months.

Notably, patients were included in the cohort based on the clinical suspicion only. Fulfillment of the EULAR definition of arthralgia at risk

for RA[33] was not required, but the different items were collected and the definition could be applied in retrospect.

Outcomes measures in RA

RA is a heterogeneous disease and its course and outcomes are highly variable between patients. For instance, it has been suggested that ACPA-positive RA and ACPA-negative RA are two different disease subsets.[37,38] The presence of ACPA is considered to be associated with worse prognosis[39–41], although this differences seems to be diminishing in recent years.[42] Notwithstanding, whereas some patients may experience a mild disease course, other patients will suffer from severe and disabling inflammation of the joints. Accurate prediction of whom will suffer from a severe or mild disease course is still inaccurate.[43,44] Monitoring disease activity in RA is one of the rheumatologist's core tasks. Several measures exist to assess the disease outcomes in RA.

First and foremost is the Disease Activity Score (DAS) which is a composition of tender and swollen joint counts, a patient-reported global assessment of disease activity as well as the level of acute phase reactants.[45] Another measure is functional ability, which is measured by the Health Assessment Questionnaire Disability Index (HAQ-DI).[46,47] The HAQ-DI consists of 20 questions in eight categories of functioning: dressing, rising, eating, walking, hygiene, reach, grip, and usual activities.[48] Scores for each category consist of a scale that is scored 0–3, representing normal (no difficulty: 0), some difficulty (1), much difficulty (2), and unable to do (3).[48] The median HAQ score for patients presenting with RA is generally 1.0.[49]

Progression of RA can also be monitored through assessment of X-rays of joints.[50] Radiographs provides a permanent record of evaluation of joint damage through which the outcome of RA can be longitudinally analysed. For research purposes, several scoring methods exist, of which the Sharp/van der Heijde method as proposed in 1989 is most commonly used.[50]

Although permanent deformities are become increasingly rare in RA, evaluating articular bone erosions can still provide key information on the burden of disease and its state of both long- and short-term activity.

Other studies have demonstrated that local subclinical inflammation as observed by imaging modalities may already be present during the earliest phases of RA.[51–55] MRI can be used to depict inflammation of the synovium of the joints (synovitis) as well as the tendons (tenosynovitis) and edema in the bone marrow (bone marrow edema; BME) and function as an outcome measure. In current research, the most used scoring method is the validated semi-quantitively scoring methodology as developed by Outcome Measures in Rheumatology Clinical Trials (OMERACT): the Rheumatoid Arthritis MRI Scoring system (RAMRIS).[35,36]

Aims of this thesis

This thesis will focus on unravelling the earliest disease phase of RA. As previous studies in autoantibody positive individuals at risk for RA have reported rates of progression around 30–50%,[29,52,56–58], there remains a need for more accurate methods to recognise imminent RA. Furthermore, understanding the burden of disease in the earliest phases of RA is required as this phase is increasingly significant due to the burgeoning interest in this phase. Finally, understanding the processes involved and affected in the early phases of RA might reduce the lacunae in current knowledge of development of RA and necessitate longitudinal studies.

The aim of this thesis is to unravel and decipher the early phases of RA and its concomitant characteristics and burden of disease.

Outline of this thesis

Part 1: Predicting progression to RA

The thesis will focus on patients with Clinically Suspect Arthralgia

identified in secondary care. Importantly, early identification of patients at risk for inflammatory arthritis and RA in secondary care is only possible if such patients are recognized and referred by GPs. Although GPs realize the need of early identification and despite the fact that national and international guidelines recommend prompt referral of patients presenting with inflammatory arthritis (IA), GPs feel uncertain in their proficiency to detect synovitis through joint examination. Our objective in c**hapter 2** was to develop and validate a rule composed of clinical characteristics to assist GPs and other physicians in identifying IA.

As mentioned in the Introduction section of this thesis, autoantibody testing is helpful for predicting the risk of progression to clinical arthritis in subjects at risk. However, most previous longitudinal studies selected autoantibody-positive arthralgia patients, and consequently the predictive values of autoantibodies were evaluated relative to one another. In **chapter 3**, the risk of individual autoantibodies, autoantibody combinations and levels for arthritis development CSA was studied, which also had an autoantibody-negative reference group. In addition to this chapter, we also investigated the implications of screening for two or three autoantibodies in persons at risk for RA in **chapter 4**.

Functional limitations in daily life in patients with Clinically Suspect Arthralgia were investigated in **chapter 5**. It is still unknown to what extent patients with arthralgia at risk for RA experience functional disability, despite the large impact of functional disability on quality of life.

Part 2: Disease mechanisms involved in progression from CSA to RA

Occurrence of structural damage (bone erosions) is one of the hallmarks of progressive disease in RA. Recent *in vitro* and murine studies indicate that

ACPA can directly activate osteoclasts leading to bone erosions and pain. The study in **chapter 6** sought evidence for this hypothesis in humans and evaluated whether in the earliest phases of RA, ACPA is associated with erosions (detected by MRI) independent of inflammation and/or RF.

Subclinical inflammation, detected by MRI, in patients with arthralgia is predictive for development of inflammatory arthritis. However, within patients that develop IA, the course of inflammation at joint level during this transition is unknown. **Chapter 7** assessed progression of inflammation at joint level.

Furthermore, the time course in which bone marrow edema, synovitis, and/or tenosynovitis (the inflammatory features that can be visualized using MRI) progress is unsettled. The longitudinal study in **chapter 8** evaluated the course of MRI-detected subclinical joint inflammation during progression to RA.

Finally in **chapter 9**, our objective was to determine the course of joint symptoms and mutual time relationships with MRI-detected subclinical inflammation in CSA patients that did not progress to RA.

Chapter 10 provides a summary of the thesis and formulates the general conclusion of the studies performed as well as general providing future perspectives. In **Chapter 11**, the summary and general conclusions are provided in Dutch.

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Part I

Predicting progression to RA

Chapter 2

Development and validation of a clinical rule for recognition of early inflammatory arthritis

R.M. ten Brinck* [1], B.T. van Dijk* [1], H.W. van Steenbergen [1], S. le Cessie [2], M.E. Numans [3], S.L. Hider [4,5], C. Mallen [4], A.H.M. van der Helm–van Mil [1]

* both contributed equally

1. Department of Rheumatology, Leiden University Medical Centre, Leiden, The Netherlands.
2. Department of Epidemiology and Statistics, Leiden University Medical Centre, Leiden, The Netherlands.
3. Department of Public Health and Primary Care, Leiden University Medical Centre, Leiden, Netherlands.
4. Research Institute for Primary Care and Health Sciences, Keele University, Newcastle, United Kingdom.
5. Haywood Academic Rheumatology Centre, Haywood Hospital, Stoke-on-Trent, Staffordshire, United Kingdom.

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Abstract

Objectives: National and international guidelines recommend prompt referral of patients presenting with inflammatory arthritis (IA), but general practitioners (GPs) feel uncertain in their proficiency to detect synovitis through joint examination, the method of choice to identify IA. Our objective was to develop and validate a rule composed of clinical characteristics to assist GPs and other physicians in identifying IA when in doubt.

Design: Split-sample derivation and validation study.

Setting: The Leiden Early Arthritis *Recognition* Clinic (EA*R*C); a screening clinic for patients in whom GPs suspected but were unsure of the presence of IA.

Participants: 1,288 consecutive patients visiting the EA*R*C .

Primary and secondary outcome measures: Associations of clinical characteristics with presence of IA were determined using logistic regression in 644 patients, while validating the results in the other 644 patients (split-sample validation). To facilitate application in clinical practice, a simplified rule (with scores ranging 0 to 7.5) was derived and validated.

Results: IA was identified by a rheumatologist in 41% of patients. In univariable analysis, male gender, age ≥60 years, symptom duration <6 weeks, morning stiffness >60 minutes, a low number of painful joints (1-3 joints), presence of patient-reported joint swelling, and difficulty with making a fist were associated with IA in the derivation dataset. Using multivariable analysis, a simplified rule consisting of these seven items was derived and validated, yielding an Area Under the Receiver Operator Characteristic curve (AUC) of 0.74 (95%CI 0.70-0.78) in the derivation dataset. Validation yielded an AUC

of 0.71 (95%CI 0.67-0.75). Finally, the model was repeated to study predicted probabilities with a lower prevalence of inflammatory arthritis to simulate performance in primary care settings.

Conclusions: Our rule, composed of clinical parameters, had reasonable discriminative ability for IA and could assist physicians in decision-making in patients with suspected IA, increasing appropriateness of health care utilization.

Background

Early initiation of disease modifying anti-rheumatic drugs is strongly associated with improved outcomes of rheumatoid arthritis (RA).[1] National and international guidelines attempt to facilitate this by emphasizing prompt referral of patients presenting with inflammatory arthritis (IA) to a rheumatologist. The European League Against Rheumatism (EULAR) taskforce for the management of early IA recommends referral within 6 weeks of onset of symptoms[2], while in the United Kingdom (UK) the National Institute for Health and Care Excellence (NICE) guidelines advises referral to a rheumatologist in patients with new, persistent (>3–4 weeks) synovitis within three working days.[3] However, it was demonstrated that this referral timeline is achieved in only 17% of patients.[4] On average, RA patients are seen four (and sometimes more than eight) times by general practitioners (GPs) before they refer to secondary care[5-8], which may reflect the difficulty of differentiating patients with early IA from patients with other types of common musculoskeletal symptoms. A recent qualitative study revealed that GPs acknowledge the importance of early detection and referral, but feel uncertain in their proficiency to detect synovitis through joint examination, the method of choice to identify IA.[2,9] As a consequence, the referral to a rheumatologist may be delayed, which contributes to overall treatment delay in early RA, as observed in Europe.[10,11]

This is further complicated by the high incidence of consultations for various common musculoskeletal symptoms and the low incidence of early IA in primary care.[12] The consultation prevalence of any musculoskeletal symptom in primary care in the UK approximates 2405 per 10,000 per year [13], making it the most common organ system consulted for at GP practices.[12-14] Although musculoskeletal symptoms are common, GPs suspect IA (based on pattern recognition) in only a very small minority of patients.[5] In these patients, GPs often lack confidence in joint assessment for synovitis.

To support early detection, several initiatives have been developed, including triage systems. The best studied triage system (the Early Inflammatory Arthritis Questionnaire) was developed and validated for patients attending secondary and tertiary care.[15-17] Furthermore, several referral guidelines for GPs[6,18-22], and public awareness campaigns have been developed, for instance one attempting to simplify pattern recognition to the "S-Factor": Stiffness, Swelling, Squeezing. However, none of these initiatives were designed using primary care data, and all assume that GPs can differentiate between the presence and absence of joint swelling[6,18-20], which continues to be a barrier to the early detection of IA.

Altogether there is a contradiction with the need to refer as quickly as possible while evidence who must be referred or, in line with this, in whom additional investigations are appropriate is lacking. To solve the issue, we have developed and validated a rule composed of clinical characteristics, by taking advantage of data from a setting intermediate between primary and secondary care. This intermediate setting of an Early Arthritis *Recognition* Clinic was a local solution to promote early referrals and is not easy implementable in other regions. The clinical rule derived from these data however, is easy to apply and may assist in the decision-making process in patients with musculoskeletal symptoms with suspected IA at other places, in order to promote early identification of IA.

Methods

Study population

To promote early recognition of early IA, the Early Arthritis *Recognition* Clinic (EA*R*C) was initiated in September 2010 in Leiden, the Netherlands. The outpatient clinic of the department of Rheumatology of the Leiden

University Medical Centre (LUMC) is the only referral centre in a healthcare region of ~400,000 people. GPs were instructed to refer patients to the EA*R*C in whom they were unsure about the presence of IA (instead of a 'wait-and-see' approach or performing additional tests). The EA*R*C system has reduced referral delay from 8 to 2 weeks, and improved early identification of IA.[11,23] To emphasize the importance of early identification of IA and aiming to inform on the purpose of the EA*R*C, a region-wide educational campaign was conducted among regional GPs.

In addition to (and distinct from) the EA*R*C, the LUMC also has an Early Arthritis Clinic (EAC). The EAC was established in 1993 to include and follow patients with early arthritis and to offer the possibility of rapid access to rheumatology care, usually within a week of referral. To differentiate between the clinics, GPs were instructed to refer to the EAC if there was a clear synovitis or very high suspicion of IA (i.e. to continue as they had before, since there was no benefit for such patients to go the EA*R*C first) and to refer to the EA*R*C when in doubt about the presence of IA (i.e. to not 'wait-and-see' or order additional tests). Thus, patients included in this study represent the difficult group in whom GPs were uncertain of the presence of suspected IA; patients with a very high degree of suspicion were referred directly to the EAC.

The EA*R*C screening clinic was held twice a week between 2010–2014 and once a week from 2014 onwards. After GP referral, patients can visit the EA*R*C without an appointment. All patients that visited the EA*R*C between 2010 and September 2015 were studied.

Data collection

At the EA*R*C, patients completed a short questionnaire about their joint symptoms, after which they were seen by an experienced rheumatologist (AvdHvM or other senior rheumatologists) who performed a full 66-joint

examination. If synovitis was determined by physical examination, patients were fast-tracked to visit the EAC within 1 week for further evaluation and treatment. Patients without IA were discharged to primary care. The questionnaire completed by patients, provided in S1 Appendix, contained questions on age, gender, date of symptom onset, date of first visit to GP, presence of a (sub)acute symptom onset (versus a gradual symptom-onset), morning stiffness (duration in minutes), which part of the day symptoms were worst, and whether they had difficulty with making a fist. Patients were asked to indicate on a 52-joint mannequin which joints were painful and which joints they considered to be swollen. IA, defined as synovitis confirmed by the rheumatologist at physical examination, was used as outcome.

Collected data was anonymized and entered in a research database at chronological order of visiting the EAC. The local medical ethical committee approved this study.

Derivation and validation of the model

We used half of the dataset for derivation and the other half for validation of results (split-sample validation). To prevent bias by (unknown) effects of inclusion period, patients with odd ID-numbers (1,3,etc) were included in the derivation dataset and those with even ID-numbers (2,4,etc) were used for validation.

To prevent exclusion of patients with one or more missing variables, we imputed missing values using chained equations[24]; frequencies of missing variables are presented in S2 Appendix. The variables 'difficulty with making a fist' and 'self-reported joint swelling' were most frequently missing as these were added to the questionnaire after April 1st 2012, thus absence of these data was considered to occur completely at random.

We conducted logistic regression analysis modelling with presence of IA (defined as rheumatologist-confirmed synovitis on physical examination) as dependent variable. Continuous variables were categorized using clinically relevant cut-offs: age: <40 / 40–59.9 / ≥60 years; duration of symptoms: <6 / 6–11 / 12–51.9 / ≥52 weeks; duration of morning stiffness: ≤60 / >60 minutes; number of painful joints: 0 / 1–3 / 4–10 / ≥11; number of swollen joints: 0 / 1–3 / 4–10 / ≥11. We performed univariable logistic regression to evaluate associations between dependent variables and presence of IA. Variables with p-values <0.05 in univariable analyses were entered in multivariable regression analyses (enter model) to obtain a model with a small number of variables. If several categories within a variable had similar regression coefficients in multivariable modelling, we pooled these categories and repeated the analysis. In sub-analysis, we also performed a multivariable logistic regression model with the pooled categories using backward selection.

To obtain a simplified rule applicable in daily care, we rounded the regression coefficients of the final multivariable logistic regression model to the nearest 0.5 (irrespective of p-value). This resulted in an easily calculable risk score. For each value of the risk score, we determined test characteristics (i.e. sensitivity and specificity) and predicted probabilities of the presence of inflammatory arthritis.

We evaluated the overall discriminative ability of the models using the Area Under the Receiver Operating Characteristic curve (AUC). The model's calibration was assessed by generating a calibration plot to measure goodness of fit, where the data was partitioned in 10 equally sized groups based on the predicted probabilities using the final fitted multivariable model. In each group, the average predicted probability on current IA was compared with the observed prevalence, both in the derivation and validation dataset. Additionally, the Hosmer-Lemeshow statistic was calculated.

To estimate performance of our simplified rule in a setting with a different prevalence of IA (e.g. primary care), a simulation was performed. Accurate data on prevalence of IA in GP practices is lacking, and therefore an estimation was made based on previous literature. One study revealed that 27% assigned with the International Classification of Primary Care-1 code for suspected IA in their medical record had confirmed RA (n=38), polyarthritis (n=5), or oligoarthritis (n=8) following rheumatologist's assessment. Another study among GPs found that 18% of patients with suspected IA was referred; though data on rheumatologists' diagnoses was not provided.[25] Guided by these scarce data obtained in GP practices, performance of the model was simulated with an estimated prevalence of 20%.[5] The intercept of the regression model was adjusted as described in [26,27] and we plotted average estimated predicted probabilities against the regression and simplified risk score. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 23.0). P-values <0.05 were considered significant.

Patient involvement

Patient research partners agreed with the pathway of care at the EA*R*C. They also provided feedback on the questionnaire, which was expanded in 2012 with two questions.

Results

Patients

1,288 patients in whom GPs were unsure about the presence of IA visited the EA*R*C between 2010 and 2015; of these, 41% had synovitis at joint examination. The frequency of inflammatory arthritis was stable throughout the study years (S3 Appendix). Baseline characteristics of patients in both derivation and validation dataset are presented in Table 1.

Model derivation

In univariable analyses, male gender, age ≥60 years, symptom duration of <6 weeks, an acute onset of symptoms, morning stiffness >60 minutes, a low number of painful joints (1–3 joints), presence of patient-reported joint swelling (1–3 joints), and difficulty with making a fist were associated with the presence of IA in the derivation dataset (Table 2). 'Symptoms worst in the early morning' was not associated with IA and therefore not included in multivariable analysis. Two multivariable models were created with categorized variables; first a model with categories similar to the univariable analysis (Table 3, model 1), and secondly a model pooling categories per variable with similar regression coefficients (Table 3, model 2). Performing this second model in the derivation dataset revealed that male gender, age ≥60 years, symptom duration of <6 weeks, a low number of painful joints (1–3 joints), and presence of patient-reported joint swelling were independently associated with the presence of IA (Table 3). The AUC of model 2 was 0.75 (95%CI 0.70–0.79) in the derivation dataset. In sub-analysis, model 2 was repeated with a backward selection procedure, showing similar regression coefficients (S4 Appendix).

Generation of a simplified rule

In order to facilitate usage in routine clinical practice, a simplified model was generated (S5 Appendix). The obtained regression coefficient of acute onset of symptoms in multivariable modelling was -0.015, yielding 0 points. Also after exclusion of this variable, the regression coefficients of the other seven variables in the model did not change yielding similar points. This resulted in a simplified rule consisting of seven scored items and a total score ranging from 0 to 7.5 with corresponding predicted risks (Figure 1). Risks of IA predicted by the model as a function of the regression score (i.e. the sum of the regression coefficients times the value of the corresponding covariates) are presented in Figure 2A; as shown, simplification did not

majorly affect the predicted risks. The calibration plot shows that predicted probabilities correlated well with the observed proportions of patients with IA (S6 Appendix). The Hosmer-Lemeshow test for the derivation dataset yielded a P-value of 0.36. If cut-offs are required and a highly sensitive approach is preferred (>90% sensitivity), this is obtained by a cut-off score of ≥4. When a highly specific approach is preferred (>90% specificity), this is obtained by a cut-off score of ≥6. Test characteristics for all cut-off points are presented in S7 Appendix. The AUC of the simplified score, measuring discrimination, was 0.74 (95%CI 0.70–0.78; S8 Appendix).

Validation

The final multivariable model (model 2) was applied in the validation dataset, revealing similar results (Table 3). The AUC was 0.72 (95%CI 0.68–0.77). Figure 2A shows the predicted probabilities of the simplified rule are almost similar to those obtained in the derivation data. The AUC of the simplified rule was 0.71 (95%CI 0.67–0.75) in the validation dataset. The calibration plot is shown in S6 Appendix, the Hosmer-Lemeshow test for the validation dataset yielded a P-value of 0.43.

Simulation of accuracy in a setting with a lower prevalence of IA

In contrast to test characteristics, predicted probabilities depend on the prior risk (i.e. prevalence) of IA. The frequency of IA among primary care patients with GP-determined clinical suspicion of IA may be different than that observed in the EA*R*C. Based on observations in GP practices[5,25], a simulation was run for the regression and simplified score with a prevalence of inflammatory arthritis set at 20%. Estimated predicted probabilities for different scores of the multivariable model and simplified rule (in derivation and validation datasets) are presented in Figure 2B.

Our simplified rule was implemented in a web application that provides predictions on the presence of current IA for individual patients; a screenshot is presented in Figure 3. The web application is accessible online at http://caretool.eu/

Discussion

GPs play a crucial role in the early identification of RA and often lack confidence in detecting joint synovitis.[9] In an attempt to solve the contradiction between the need to refer very early and absence of evidence who must be referred, we provided an evidence-based and simple method to identify the presence of IA in patients in whom IA is suspected. This clinical rule helps to select patients to refer for additional investigations (laboratory or imaging) or to secondary care. Hence, the Clinical Arthritis RulE could increase appropriateness of health care utilization.

This study is different from studies that derived tools to facilitate triage of patients that have been referred to secondary or tertiary care[15-17] as our study did not aim to prioritize patients that are already referred. In addition, we aimed to facilitate recognition of IA (as this would necessitate prompt referral to a rheumatologist) and did not perform a longitudinal study to predict development of specific diagnoses (e.g. RA) later-on. This explains why several factors were found to be associated with presence of IA that are not generally considered typical for RA (male gender, a low number of painful joints, a short symptom duration). GPs generally do well in identifying those at high risk for development of RA (i.e. women with subacute smouldering polyarticular, symmetric complaints), and therefore we aimed this tool to assist GPs in decision-making for more atypical or non-classical presentations of IA (e.g. due to overlap of symptoms with other diagnoses) leading to doubt. Indeed, many of the patients that did not have synovitis at the EA*R*C had symptoms due to diagnoses that are

characterized by longstanding or extensive joint pain (e.g. osteoarthritis, fibromyalgia), explaining higher scores for a short symptom duration or a low number of symptomatic joints.

Adding other clinical variables might increase the discriminative ability of the model. Potential examples include the squeeze test of the metacarpophalangeal joints (although the diagnostic accuracy was shown to be only moderate[28]), information on family history, or functional impairments. These items were not routinely collected before December 2015. Adding data on laboratory investigations to our rule could potentially also increase its discriminative ability. However, our data do not permit us to evaluate this, as additional investigations were done afterwards and only in patients with synovitis at joint examination.

A strength of our EA*R*C for the purpose of this study is that GPs in our region are familiar with the need for early referral and that regional healthcare logistics make rheumatology care rapidly available for patients with arthritis, with the EA*R*C as ultimate service for patients in whom GPs suspect (but are unsure about) IA. With the availability of the EA*R*C every week and lack of any waiting list for the EA*R*C, we assume a low number of patients not showing up at the EA*R*C despite being encouraged by their GP to visit the EA*R*C. As the EA*R*C serves as a unique bridge between primary and secondary care, its patients closely resemble the population GPs have contact with and have doubts about. Although the EA*R*C is successful in our region[11,23], this approach may be more difficult to implement in other centres or regions due to a shortage of rheumatologists, or long traveling distances to rheumatology outpatient clinics, and as such a different system is needed to aid GPs in identifying IA. This prompted us to derive a validated rule composed of clinical characteristics that could assist GPs in decision-making for more atypical or non-classical (but nevertheless suspect) presentations of IA, as classical presentations usually don't cause GPs concern.

GPs were discouraged (both by our local communication with GPs and according to national guidelines for GPs) to perform autoantibody testing.[29] Autoantibody testing in primary care in this region was infrequent[5], unlike in other parts of the world. Autoantibody testing may falsely reassure doctors and patients, especially when results are negative, and as such we believe a model based on clinical presentation is more appropriate to facilitate rapid referral.

Another strength is that we studied patients in whom the GPs have indicated a lack of confidence to identify the presence of synovitis. Patients with clinically obvious IA had early access to rheumatologic care already. This may enhance the generalizability of the present data to the setting of doubt in primary care. Furthermore, the use of real-life observational data in our study may boost external validity of the results.

A disadvantage of our setting is that the data were not collected in primary care itself, but in a setting intermediary between primary and secondary care. Although musculoskeletal symptoms are a very common reason for consulting primary care, suspected IA is relatively unusual, and the average full-time GP diagnoses only one new patient with RA each year.[30] Additionally, although the EA*R*C is easily accessible on a weekly basis, the exact number of patients that were referred but did not visit the EA*R*C is unknown. Validation in primary care is required. We studied 'the difficult group' of patients in whom GPs were uncertain of the presence of suspected IA. The prevalence of such patients in primary care may be higher and as a consequence the actual prevalence of IA among suspected IA patients may be lower than 41% in primary care. Since the post-test probabilities strongly depend on the prevalence (i.e. pre-test probability), a simulation was performed with an estimated prevalence of IA that was half of the prevalence as observed in our data (20%). The choice of 20% was based on literature from primary care; although not much is known about

suspected IA in primary care, two study suggested a prevalence of IA among suspected patients of 18-27%[5, 25]. We demonstrated the predictive accuracy of the model using a simulated prevalence of 20%. Because of the limitation that no other data are available on the prevalence of IA when GPs suspect IA, this estimated prevalence could be an overestimation. However, the observed data could also be an underestimation as in our setting GPs were instructed to refer patients with high suspicion/definite arthritis to the regular outpatient clinic. Further external validation in GP settings is therefore required.

GPs in our region are well informed about the importance of the early detection of IA, but the GPs in our region feel that their actual detection skills are not different from that of GPs elsewhere. However if the detection skills of our GPs are different from that of GPs in other regions, a lower prevalence of IA (and therefore lower pre-test probabilities) may be present. As a consequence, the rule may yield lower post-test probabilities. This effect may have been dealt with in the simulation analysis but still external validation in primary care and preferably in different regions or countries is necessary.

We expect that our rule (Clinical Arthritis RulE - CARE) might support GPs and other health care professionals in the decision-making process in patients with musculoskeletal symptoms in whom they suspect IA, regardless of the region. Of course, the consequences of an increased score will likely depend on the setting and relation with secondary care: it can either influence the decision to directly refer a patient or to first ask for additional laboratory tests (e.g. acute phase reactants or autoantibodies; Figure 4). A clinical decision aid may be of value to this end as well, as for most laboratory investigations the diagnostic accuracy depends on the prior risk. Using a simple clinical decision aid first may be more cost-effective than performing additional investigations in all patients in whom there is doubt about IA. Depending on the setting and consequences

of a high score, either a sensitive method or a specific method may be preferred; for this reason cut-offs for both situations are provided. The web application, also easily assessable by phone, facilitates implementation of the Clinical Arthritis RulE by GPs, physicians, and other health care professionals such as physiotherapists in their daily work.

Conclusion

In conclusion, this study developed a clinical rule that supports the identification of patients suspected of having IA by physicians that feel insufficiently experienced in assessment of synovitis by joint examination. We hope the current data are a prelude to a data-driven method that supports GPs, physicians, and other health care professionals in decision-making in patients with suspected early IA.

Supporting information

Supplementary data is available at the website of BMJ Open, or can be obtained by contacting the first author.

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Table 1. Characteristics of patients visiting the Early Arthritis *Recognition* Clinic

	Derivation (N=644)	Validation (N=644)	P-value**
Male, n (%)	190 (30)	198 (31)	0.62
Age in years, mean ± SD	52 16	51 17	0.27
Symptom duration in weeks, median (IQR)	10 (3–45)	12 (4–45)	0.18
Acute onset of symptoms *, n (%)	252 (39)	238 (37)	0.45
Symptoms worst in the early morning, n (%)	372 (58)	351 (55)	0.10
Morning stiffness in minutes, median (IQR)	10 (0–30)	10 (0–30)	0.33
Number of painful joints, median (IQR)	7 (2–15)	6 (3–15)	0.69
Number patient-reported swollen joints, median (IQR)	2 (1–5)	2 (1–5)	0.19
Difficulty with making a fist, n (%)	329 (51)	301 (47)	0.06
Arthritis present at joint examination by experienced rheumatologist, n (%)	271 (42)	252 (39)	0.28

Legend:
* Patients were asked to define onset of symptoms; either acute onset of symptoms or gradual onset of symptoms, see S1 Appendix. Abbreviations: IQR = interquartile range; SD = standard deviation. ** Unpaired t-tests, chi-squared tests and Mann-Whitney U tests were used as appropriate.

Table 2. Univariable logistic regression in the derivation dataset with presence of synovitis upon joint examination as outcome.

		Arthritis (N=271)	No artritis (N=373)	OR (95% CI)
Male, n (%)		104 (38)	86 (23)	2.1 (1.5–2.9)
Age, n (%)	<40	49 (18)	104 (28)	*(ref)*
	40–59.9	109 (40)	172 (46)	1.3 (0.89–2.0)
	≥60	113 (42)	97 (26)	2.5 (1.6–3.8)
Symptom duration in weeks, n (%)	<6	124 (46)	103 (28)	3.8 (2.4–5.9)
	6–11	38 (14)	62 (17)	1.9 (1.1–3.9)
	12–51.9	66 (24)	75 (20)	2.7 (1.7–4.5)
	≥52	43 (16)	132 (36)	*(ref)*
Acute onset of symptoms *, n (%)		122 (45)	131 (35)	1.5 (1.1–2.1)
Symptoms worst in early morning, n (%)		158 (58)	214 (57)	1.1 (0.69–1.6)
Morning stiffness >60 min, n (%)		45 (17)	40 (11)	1.7 (1.03–2.7)
Number of painful joints, n (%)	0	1 (0)	10 (3)	*(ref)*
	1–3	110 (41)	82 (22)	13.2 (1.7–105.5)
	4–10	76 (28)	123 (33)	6.1 (0.77–49.0)
	≥11	84 (31)	158 (42)	5.2 (0.65–41.3)
Number of patient-reported swollen joints, n (%)	0	18 (7)	71 (19)	*(ref)*
	1–3	115 (42)	119 (32)	3.7 (2.0–6.9)
	4–10	87 (32)	115 (31)	2.9 (1.5–5.5)
	≥11	51 (19)	68 (18)	2.9 (1.4–5.9)
Difficulty with making a fist, n (%)		156 (58)	172 (46)	1.6 (1.1–2.4)

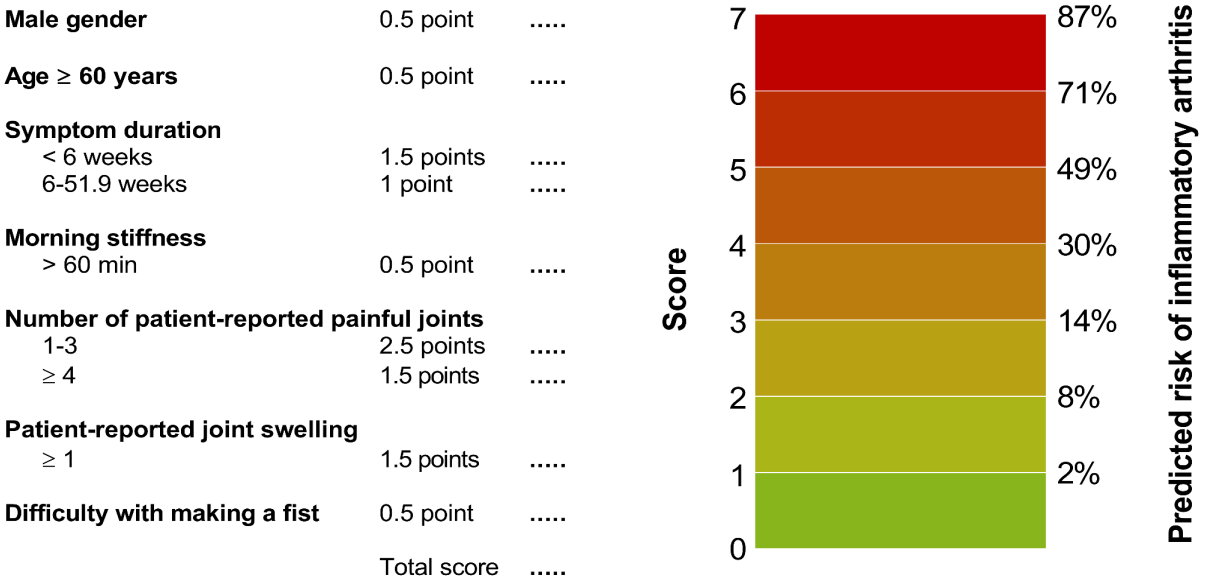
Legend:
* Patients were asked to define onset of symptoms; either acute onset of symptoms or gradual onset of symptoms, see S1 Appendix. Abbreviations: CI = confidence interval; OR = odds ratio.

Table 3. Multivariable logistic regression analyses with synovitis upon joint examination as outcome.

		Model 1		Model 2		
		Derivation		**Derivation**		**Validation**
		OR (95% CI)		**OR (95% CI)**	**B**	**OR (95% CI)**
Male		1.7 (1.1–2.5)		1.7 (1.1–2.5)	0.517	1.7 (1.1–2.4)
Age (years)	<40	*(ref)*	0–59.9	*(ref)*	(*ref*)	*(ref)*
	40–59.9	1.5 (0.96–2.5)	≥60	2.1 (1.4–3.1)	0.750	2.1 (1.5–3.0)
	≥60	2.9 (1.7–4.8)				
Symptom duration (weeks)	<6	3.8 (2.3–6.4)	<6	3.6 (2.2–6.0)	1.279	3.4 (2.0–5.7)
	6–11	1.7 (0.92–3.1)	6–51.9	2.2 (1.4–3.6)	0.797	1.9 (1.2–3.0)
	12–51.9	2.9 (1.7–5.0)	≥52	*(ref)*	(*ref*)	*(ref)*
	≥52	*(ref)*				
Acute onset of symptoms*		1.0 (0.67–1.5)		0.99 (0.66–1.5)	-0.015	1.0 (0.70–1.5)
Morning stiffness (minutes)	>60	1.6 (0.88–2.9)	>60	1.6 (0.91–2.9)	0.485	1.2 (0.62–2.3)
Number of painful joints	0	*(ref)*	0	*(ref)*	(*ref*)	*(ref)*
	1–3	9.3 (1.1–78.2)	1–3	10.0 (1.2–83.4)	2.300	7.9 (0.91–68.6)
	4–10	4.5 (0.53–37.6)	≥4	4.5 (0.54–37.1)	1.497	5.2 (0.61–45.1)
	≥11	3.3 (0.39–28.4)				
Number of patient-reported swollen joints	0	*(ref)*	0	*(ref)*	(*ref*)	*(ref)*
	1–3	3.2 (1.6–6.4)	≥1	3.5 (1.9–6.6)	1.253	3.7 (1.9–7.0)
	4–10	3.4 (1.7–7.0)				
	≥11	4.3 (1.9–10.0)				
Difficulty with making a fist		1.6 (0.97–2.5)		1.6 (0.99–2.6)	0.467	1.4 (0.91–2.2)
Intercept		-4.8			-4.6	-4.6
AUC		0.76 (0.71–0.80)		0.75 (0.70–7.79)		0.72 (0.68–0.77)

Legend:
Model 1 includes categories of clinically applicable cut-offs; if within variables several categories had similar regression coefficients, categories were pooled (Model 2).
* Patients were asked to define onset of symptoms; either acute onset of symptoms or gradual onset of symptoms, see S1 Appendix. Variables with p-values <0.05 in univariable analysis in the derivation set were entered in multivariable regression analyses. Abbreviations: B = beta; CI = confidence interval; OR = odds ratio.

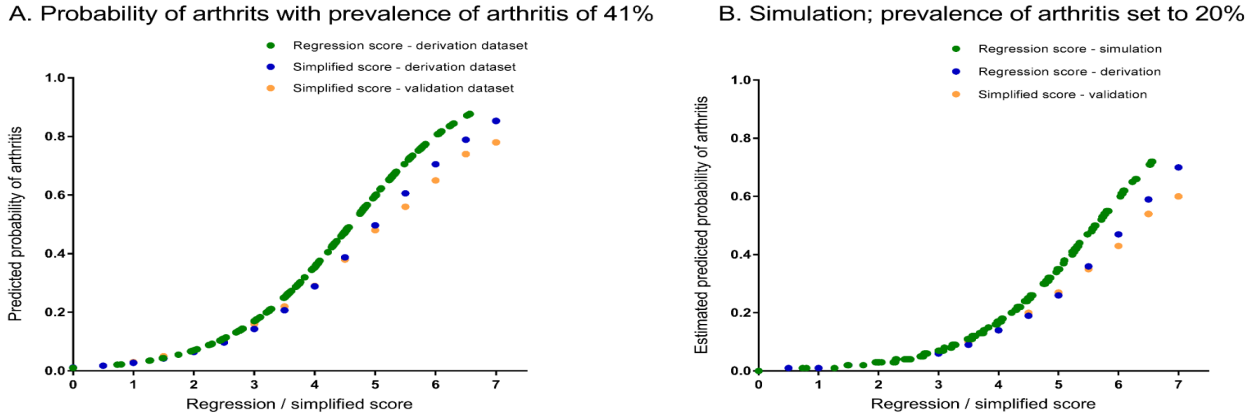
Figure 1. The Clinical Arthritis RulE (CARE) and corresponding predicted risks of presence of inflammatory arthritis per score.



Legend:

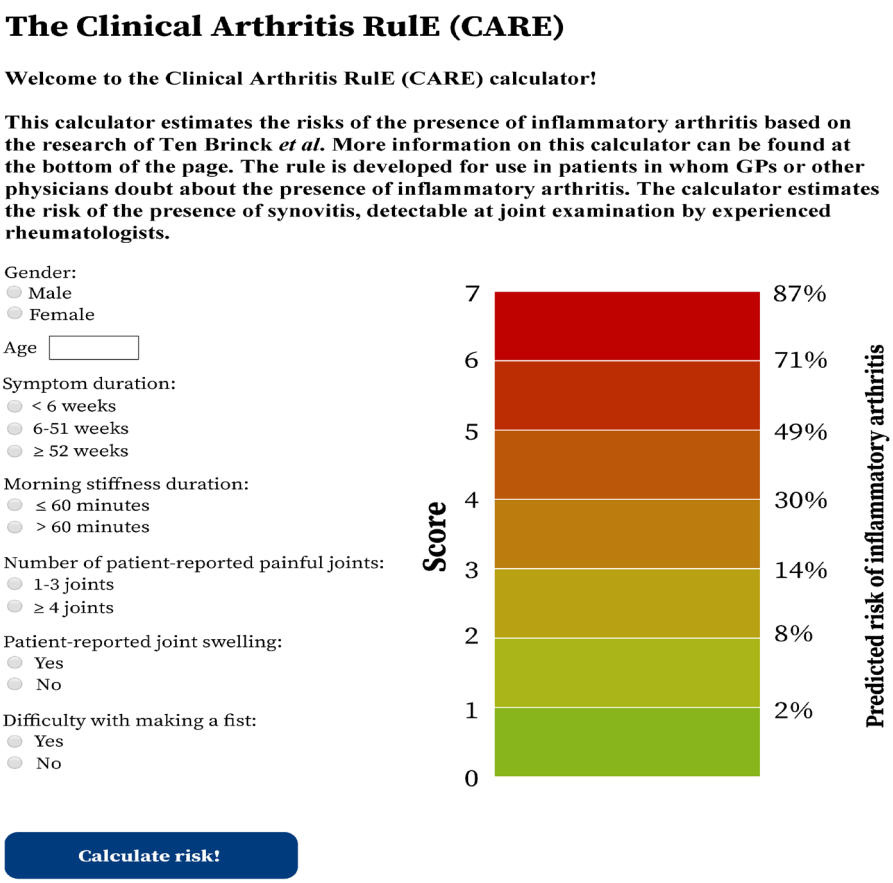
Observed risks of current inflammatory arthritis were obtained by calculating the proportion of patients with a positive outcome (rheumatologist-confirmed synovitis) for each value of the risk score in the derivation dataset.

Figure 2. The Clinical Arthritis RulE (CARE) and presentation of the predicted probabilities of the presence of current inflammatory arthritis based on the regression model, and the simplified score as observed in the derivation and validation datasets (A), and estimated predicted probabilities in a simulation with a pre-test probability (i.e. prevalence) of inflammatory arthritis of 20% (B).



Legend:
Predicted probabilities of the final multivariable logistic regression model, fitted in the derivation set as function of the regression score (i.e. the sum of the regression coefficients times the value of the corresponding covariates (green line)). Furthermore, for each value of the simplified score the mean predicted probability is plotted in the derivation and validation dataset (blue and orange dots).

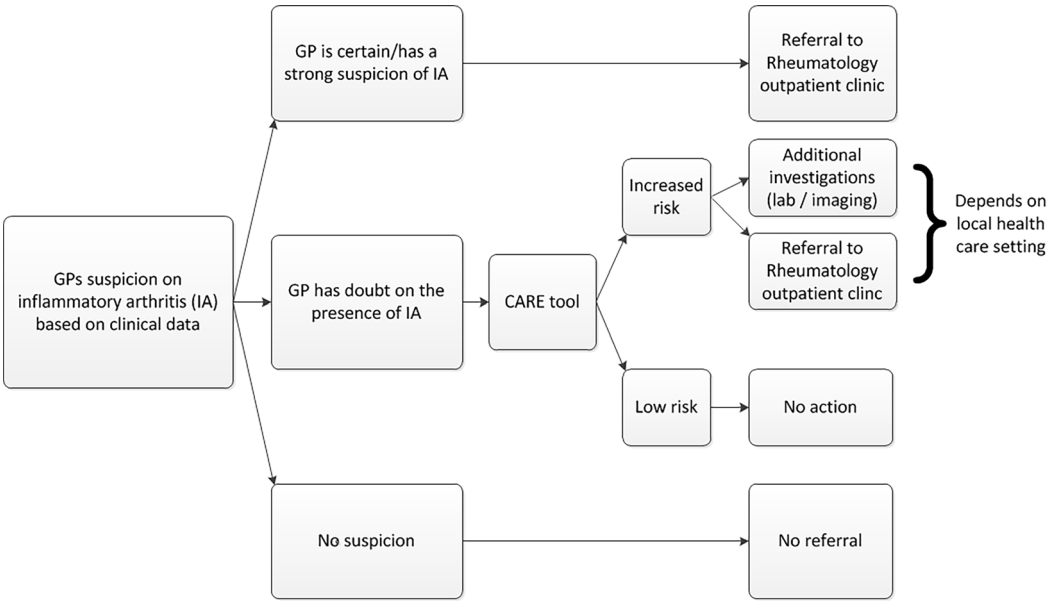
Figure 3. A stylized representation of the Clinical Arthritis RulE, to be used in patients in whom GPs doubt about the presence of inflammatory arthritis.



Legend:

The web application that provides predictions on the predicted risk of inflammatory arthritis for individual patients as can be accessed at http://caretool.eu/

Figure 4. Flowchart of decision-making in patients with suspected early IA based on clinical characteristics and the role of the Clinical Arthritis RulE.



Chapter 3

The risk of individual autoantibodies, autoantibody combinations and levels for arthritis development in Clinically Suspect Arthralgia

R.M. ten Brinck [1], H.W. van Steenbergen [1], M.A.M. van Delft [1], M.K. Verheul [1], R.E.M. Toes [1], L.A. Trouw [1], A.H.M. van der Helm–van Mil [1]

1. Department of Rheumatology, Leiden University Medical Centre, Leiden, the Netherlands.

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Abstract

Introduction: Autoantibody testing is helpful to predict the risk of progression to clinical arthritis in subjects at risk. Previous longitudinal studies have mainly selected autoantibody-positive arthralgia patients and, consequently, the predictive values of autoantibodies were evaluated relative to each other. This study assessed risks for arthritis development of anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF) and/or anti-carbamylated protein antibodies (anti-CarP) in arthralgia patients considered at risk for RA by rheumatologists based on clinical characteristics (Clinically Suspect Arthralgia, CSA).

*Methods***:** Baseline ACPA, RF and anti-CarP autoantibody-status of 241 patients, consecutively included in the CSA-cohort, was studied for risk of developing clinical arthritis during a median follow-up of 103 (IQR 81-114) weeks.

*Results***:** Univariable associations for arthritis development were observed for ACPA, RF and anti-CarP antibodies; Hazard Ratios (95%CI) were 8.5 (4.7-15.5), 5.1 (2.8-9.3) and 3.9 (1.9-7.7) respectively. In multivariable analysis, only ACPA was independently associated (HR 5.1; 2.0-13.2). Relative to autoantibody-negative CSA-patients, ACPA-negative/RF-positive patients had HRs of 2.6 (1.04-6.6), ACPA-positive/RF-negative patients 8.0 (2.4-27.4), and ACPA-positive/RF-positive patients 10.5 (5.4-20.6). Positive predictive values (PPV) for development of clinical arthritis within two years were: 38% for ACPA-negative/RF-positive, 50% for ACPA-positive/RF-negative and 67% for ACPA-positive/RF-positive patients. Higher ACPA-levels were not significantly associated with increased progression to clinical arthritis, in contrast to higher RF-levels. Autoantibody levels were stable during follow-up.

*Conclusion***:** ACPA conferred the highest risk for arthritis development and had an additive value to RF. However, >30% of ACPA-positive/RF-positive CSA-patients did not develop arthritis during two-year follow-up. Thus, CSA and information on autoantibodies is insufficient to accurately identify imminent autoantibody-positive RA.

Introduction

Anti-Citrullinated Protein Antibodies (ACPA), Rheumatoid Factor (RF) and antibodies against carbamylated proteins (anti-CarP) can be present years before the first onset of symptoms of rheumatoid arthritis (RA)[1-3]. Initial observations on the association between autoantibodies and progression to clinical arthritis were largely done in nested case-control studies[2,3]. Results of these studies cannot be directly used for risk assessment in clinical practice; longitudinal studies performed in daily rheumatologic practice are needed to this end[4-6].

Most published longitudinal studies in arthralgia determined predictive values of ACPA and RF in persons that were selected for the presence of these autoantibodies[4,5,7,8]. Consequently, as a reference group of arthralgia patients without autoantibodies was not available, predictive values of the different autoantibodies were evaluated relative to each other[4,5]. RF-positive patients were often used as reference group, as presence of RF yielded the lowest risk of progression to clinical arthritis[5]. In addition, some of the patients in these studies had musculoskeletal symptoms but were not referred to secondary care because of these symptoms[7]. The selection method and reference group used in these studies may affect generalizability for arthralgia patients presenting to rheumatology outpatients clinics. Therefore, the risks provided by (combinations of) different autoantibodies in patients presenting with arthralgia at risk for RA is still undetermined.

The present study evaluated patients with clinically suspect arthralgia (CSA); these are patients without clinical arthritis that are considered at risk of progression to RA by their rheumatologists based on the clinical presentation. Identification of patients at risk based on clinical expertise is to some extent subjective and to allow inclusion of a more homogeneous group of patients in studies, a EULAR-definition for arthralgia suspicious

for progression to rheumatoid arthritis was recently developed[9]. This definition is intended for use in arthralgia patients without clinical arthritis in whom imminent RA is considered more likely than other explanations. This will generate a more homogenous set of arthralgia patients at risk for RA and may facilitate generalizability of findings to arthralgia patients in other outpatient clinic settings.

To determine the value of RA-related autoantibodies in patients with CSA, this study aimed to: 1) Determine progression to clinical arthritis and the absolute risks provided by ACPA, RF and anti-CarP antibodies. 2) Determine the risk provided by combinations of the commercially available autoantibody-tests: ACPA and RF. 3) Evaluate if higher ACPA- and RF-levels conferred higher rates of progression to clinical arthritis. In addition, sub-analyses were performed in which we aimed to 4) Investigate differences in baseline characteristics of ACPA-positive/RF-positive patients that did and that did not progress to clinical arthritis and 5) Assess ACPA- and RF-levels over time, both in patients that progressed from CSA to arthritis and in patients that did not progress.

Methods

Patients

Two hundred and forty-one patients were consecutively included in the Leiden Clinically Suspect Arthralgia (CSA) cohort between April 2012 and March 2015, an inception cohort at the rheumatology outpatient clinic of the Leiden University Medical Centre, the Netherlands. Per definition, CSA-patients had no clinical arthritis, but recent-onset (<1 year) arthralgia of hand or feet joints and were considered at risk for RA based on the clinical expertise of the rheumatologists, as described previously[10]. Hence, patients were indicated as having CSA based on the first clinical presentation. As general practitioners in the region are discouraged to perform autoantibody testing before referral[11,12], information on ACPA- and RF-status were generally unknown at secondary care presentation.

After inclusion, questionnaires were filled by patients and rheumatologists, joint counts performed, blood samples taken, and a unilateral contrast-enhanced MRI was made of 2nd-5th metacarpophalangeal, wrist and 1st-5th metatarsophalangeal joints of the most painful side (or dominant side in case of equally severe symptoms at both sides) using an MSK-extremity 1.5T MRI-scanner as described elsewhere[10,11] and in the Supplementary Methods. Regular follow-up visits were scheduled at 4,12 and 24 months and additional visits occurred in between if indicated (either if felt necessary by rheumatologists or at request of patients because of an increase in symptom severity). Treatment with Disease-Modifying Antirheumatic Drugs (DMARDs) was not allowed during the CSA study, NSAIDs were allowed. The CSA-cohort has been approved by the local medical ethical committee (named "Commissie Medische Ethiek"). All participants provided written informed consent according to the declaration of Helsinki.

Autoantibody determination

At baseline visit, Immunglobulin-G ACPA (EliA CCP (anti-CCP2), Phadia, Nieuwegein, the Netherlands), Immunoglobulin-M RF (as described previously, in-house ELISA[13]), and Immunoglobulin-G anti-CarP antibodies were determined. The cut-off for ACPA-positivity was >7 U/mL; for RF-positivity it was >3.5 IU/mL, according to the manufacturer's instructions. ACPA- and RF-status were repeated after two years, or at the time of conversion to clinical arthritis. Anti-CarP was determined as described previously[14]. As no commercial kit is available for anti-CarP antibodies, we have used our in-house developed anti-CarP assay based on carbamylated Fetal Calf Serum (FCS) and as a control the non-modified FCS as the coating antigens in ELISA[14]. The cut-off was equivalent to 2 Standard Deviations (SD) above the mean in a group of healthy controls. The controls consisted of a group of 197 healthy blood donors. Mean age of the controls was 44.4 years (range 20-70 years, SD 14). 50.8% of controls were female. Controls were not allowed to have a rheumatic disease. 65.5% of controls had never smoked, 26.9% had previously smoked, 6.6% were current smokers and in two controls data on smoking status was missing.

Outcome

All patients were followed for ≥56 weeks. Median follow-up duration was 103 weeks, interquartile range 81 to 114 weeks. None of the patients were treated with DMARDs or corticosteroids in the phase of CSA. Primary endpoint was development of arthritis detected at physical examination (66 joints assessed) by the rheumatologist. Medical records of all patients were studied for established clinical arthritis until April 22nd 2016. Persistent arthritis was studied as secondary endpoint (through study of the medical record), which was defined as clinical arthritis that persisted at two subsequent visits or when DMARDs were prescribed when clinical arthritis was identified. The 2010 classification criteria for RA[15] were considered less suitable as secondary outcome, as autoantibody-negative patients require >10 involved joints to fulfil these criteria[16]. DMARDs were generally started shortly after patients had developed clinically evident arthritis and this may have prevented progression from unclassified arthritis to RA, particularly for autoantibody-negative patients.

Statistical analyses

Univariable and multivariable Cox proportional hazards regression analyses were performed with clinical arthritis as outcome. Time to clinical arthritis was defined as time from inclusion date in the cohort to the date of first detection of clinical arthritis. Patients who did not develop arthritis were censored at either the date that all medical files were studied on arthritis development or at the date of the 24-month follow-up visit. When evaluating hazard ratios and absolute risks for combinations of autoantibodies, we mainly restricted ourselves to the two commercially available autoantibodies (ACPA and RF), because otherwise small subgroups would be obtained (Supplementary Figure 1). To determine the association for arthritis development with autoantibody level, patients were categorized into tertiles based on ACPA-levels of ACPA-positive patients in our cohort or RF-levels in RF-positive patients in our cohort (hence creating three groups of similar size). For ACPA, these categories

were 7-95 U/ml (with N=10), 96-325 U/ml (N=11) and ≥326 U/ml (N=11). For RF, the categories were 3.5-10 IU/ml (N=17), 11-40 IU/ml (N=17) and ≥41 IU/ml (N=17). Test characteristics and predictive values with 95% confidence intervals were calculated. Patient characteristics were compared using Mann-Whitney U tests, t-tests and χ^2 tests as appropriate.

In addition to the analyses on all CSA-patients, the most important analyses were repeated in the subgroup of patients that also fulfilled the EULAR-definition of arthralgia suspicious for progression to RA (3/7 items present)[9]. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS, version 23.0). P-values <0.05 were considered significant.

Results

Patients with CSA

Baseline characteristics of the 241 CSA-patients are shown in Table 1. During a median follow-up period of 103 (IQR 81-114) weeks, 44 patients progressed to clinical arthritis (Figure 1). The secondary endpoint was obtained in 41 patients: 3 patients had clinical arthritis on only one occasion that resolved spontaneously (without DMARD treatment) before the next visit; one patient had clinical arthritis in a wrist joint and two patients in the elbow joint.

Presence of autoantibodies and hazard ratio for progression to clinical arthritis

In univariable Cox regression, presence of ACPA was associated with arthritis development (Hazard Ratio 8.5; 95%CI 4.7-15.4). A similar observation was made for presence of RF (HR 5.1; 95%CI 2.8-9.3) or anti-CarP antibodies (HR 3.9; 95%CI 1.9-7.7). Multivariable analysis including all three autoantibodies – to correct for simultaneous presence of the autoantibodies – revealed an independent significant association for ACPA

only (HR 5.1; 95%CI 2.0-13.2); the HR for RF and anti-CarP antibodies were 2.0 (95%CI 0.81-4.9) and 1.04 (95%CI 0.46-2.4) respectively. When age, gender, smoking and positive family history for RA were also included in the multivariable model, only ACPA was significantly associated with progression to RA (HR 5.3; 95%CI 2.0-14.2).

Combinations of ACPA and RF and associated hazards

Combinations of ACPA and RF were studied next, as these are the commercially available tests and are most commonly used in daily rheumatologic care. With autoantibody-negative CSA patients as reference, ACPA-negative/RF-positive patients had a HR of 2.6 (95%CI 1.04-6.6) for developing clinical arthritis. ACPA-positive/RF-negative had a HR of 8.0 (95%CI 2.4-27.4), and ACPA-positive/RF-positive patients had a HR of 10.5 (95%CI 5.4-20.6), see Figure 2. The hazard was not significantly different between ACPA-positive/RF-negative and ACPA-positive/RF-positive patients (p=0.78), but there was a significantly different hazard ratio between ACPA-negative/RF-positive and ACPA-positive/RF-positive patients (p=0.005).

Although subgroups became small when anti-CarP was also considered (Supplementary Figure 1), there were no significant associations of anti-CarP with arthritis development within ACPA-negative/RF-negative or within ACPA-positive/RF-positive patients (HR 2.7; 95%CI 0.62–11.9 and HR 1.0; 95%CI 0.37–2.7 respectively).

Association of autoantibody levels and arthritis development

In RA, presence of multiple autoantibodies is associated with higher autoantibody levels[17,18]. In CSA-patients, higher ACPA-levels were observed in ACPA-positive/RF-positive patients than in ACPA-positive/RF-negative patients (median 237.5 U/mL versus 94 U/mL, p=0.17). Within ACPA-positive patients, ACPA-levels were not associated with higher

hazards for progression to clinical arthritis (Figure 3A). RF-levels were significantly higher in ACPA-positive/RF-positive patients compared to ACPA-negative/RF-positive patients (median 36 IU/mL versus 12.5 IU/mL respectively, p=0.007). In addition, patients with RF-levels ≥41 IU/ml (highest tertile) had significantly increased hazard to progress to clinical arthritis (HR 3.3; 95%CI 1.1-9.6) compared to patients with RF-levels 3.5-10 IU/ml (lowest tertile, Figure 3B).

Absolute risks and test characteristics for arthritis development at 2 years follow-up.

In order to arrive at absolute risks for developing clinical arthritis of individual autoantibodies and combinations of ACPA and RF, patients that completed two-year follow-up were studied (n=144). Positive predictive values (PPV) for arthritis development within two years were: 63% for ACPA, 53% for RF and 50% for anti-CarP antibodies. Considering combinations of ACPA and RF, the PPV for ACPA-negative/RF-positive patients was 38%. For ACPA-positive/RF-negative patients, PPV was 50% and for ACPA-positive/RF-positive patients 67% (Table 2). Thus, of the ACPA-positive/RF-positive patients, 33% did not develop arthritis within two years. Sub-analyses with the secondary endpoint (persistent clinical arthritis) showed almost similar results (Supplementary Table 1).

Similar findings in patients that fulfilled the EULAR-definition of arthralgia suspicious for progression to RA

178 of the 241 patients (74%) that were identified as CSA by their rheumatologists also fulfilled the EULAR-definition. The HRs for progression to arthritis within 2-years were: 2.4 (95%CI 0.89-6.5) for ACPA-negative/RF-positive patients, 5.9 (95%CI 1.4-25.8) for ACPA-positive/RF-negative patients, and 9.7 (95%CI 4.7-20.2) for ACPA-positive/RF-positive patients (Supplementary Figure 2). Predictive values and test characteristics are presented in Supplementary Table 2. Of the ACPA-positive/RF-positive patients that fulfilled the EULAR-definition, 31% did not progress to RA.

Baseline characteristics of ACPA-positive/RF-positive CSA-patients that progressed to arthritis versus those that did not.

We hypothesized that patients progressing to clinical arthritis had either higher autoantibody levels or more extended (systemic or local subclinical) inflammation than patients that did not progress. Therefore, we then explored if ACPA-positive/RF-positive CSA-patients that did not progress to arthritis during 2 years follow-up differed in baseline characteristics from those that progressed. Although the number of patients in both groups was small, no statistically significant or clinically relevant differences were observed (Table 3).

Serum levels of ACPA and RF over time

Of the 44 CSA-patients that progressed to clinical arthritis, 20 were ACPA-positive with a median ACPA-level at CSA-inclusion of 266 U/ml (IQR 130-340) and 200 U/ml (IQR 91.75-340) at arthritis development (p=0.39). Similarly, of the 44 patients that progressed, 23 were RF-positive with a median RF-level of 29 IU/ml at CSA-inclusion and 39.5 IU/ml at arthritis development (p=0.99).

Autoantibody status and autoantibody levels were also assessed in patients who had completed two-year follow-up and did not progress to clinical arthritis (N=114). Of these patients, 10 were ACPA-positive at inclusion and none of these patients changed in ACPA-status during follow-up. The median ACPA-level in these non-converting patients was 304 U/ml at baseline and 340 U/ml after 2-years. Similarly, 16 patients not progressing to clinical arthritis were RF-positive at baseline; during follow-up, one RF-positive patient became RF-negative (levels 4.3 IU/mL and 3.0 IU/mL respectively) and one RF-negative patient became RF-positive after 2 years (levels <0.4 IU/mL and 12.0 IU/mL respectively). The median RF-level in non-converting patients was 16.5 IU/ml at baseline and 11 IU/ml after 2

years. Overall, status and levels of ACPA and RF were rather stable during two year follow-up, both in patients that progressed to clinical arthritis and in patients that did not progress to clinical arthritis.

Discussion

Early recognition of patients with imminent RA is an important but challenging topic. Autoantibodies have proven to be the most powerful predictors for development of clinical arthritis currently available. This study thoroughly determined the risks of individual autoantibodies, combinations of autoantibodies and autoantibody-levels in patients that were considered at risk for RA based on their clinical presentation. The absolute risks for progression to arthritis may be useful for daily clinical practice at places where patients present with arthralgia to rheumatology outpatient clinics. We observed that ACPA, RF and anti-CarP antibodies were associated with increased risks, but that only ACPA was independently associated with development of RA in multivariable analysis. Furthermore, although ACPA was clearly additive to RF in predicting risks, vice versa, RF was less additive to ACPA.

A previous study by Van Steenbergen evaluated the risk of ACPA, but not the other autoantibodies in CSA[11]. The current study explored different characteristics of several different autoantibodies in CSA, in a larger study population and during a longer duration of follow-up. As previously described, the absolute risk of ACPA for arthritis development within two years was 63%. Previous studies in other at-risk populations found lower positive predictive values. A study in ACPA-positive patients with non-specific musculoskeletal complaints showed progression to clinical arthritis of 47% within 12 months[7]. A study in ACPA-positive and/or RF-positive arthralgia patients found a PPV of 35% during the first year[8]. Positive predictive values are dependent on enrichment (i.e. prevalence) of cases in cohort studies, meaning that the same test may yield different results

depending on the setting. Patients that are identified as having CSA by rheumatologists comprise a small group of all patients presenting with arthralgia to secondary care (<6%)[19]. This yielded higher prior chances for RA-development in CSA-patients than patients with non-specific arthralgia in secondary or primary care. Presumably, this explains the higher post-test chances of ACPA in this setting.

CSA is defined by the clinical expertise of rheumatologists and is therefore subjective. A EULAR-taskforce has recently derived a definition of arthralgia suspicious for progression to RA, in order to strip CSA from its subjectivity and to allow evaluation of a more homogeneous group of patients. Although further longitudinal studies on the accuracy of the EULAR-definition are required, the present data suggest that the clinical expertise of the rheumatologists was often in line with the EULAR-definition.

In the present data, despite small numbers, higher RF-levels were associated with an increased risk of progression to clinical arthritis. Furthermore, patients with higher RF-levels were also more often ACPA-positive. RF-positive/ACPA-positive patients had a higher risk of developing clinical arthritis than RF-positive/ACPA-negative patients. Hence, these findings are compatible with each other. ACPA-levels were not associated with a significantly increased risk of developing arthritis. This finding is in line with that of non-significant differences in the risk of developing arthritis between ACPA-positive/RF-positive and ACPA-positive/RF-negative patients, as both groups also had no significant differences in ACPA-level. However, it should be noted that subgroups of patients with different autoantibody levels were small.

This is the first longitudinal study evaluating the effect of anti-CarP antibodies in relation to RF and ACPA in CSA. A previous study observed an association of anti-CarP antibodies with arthritis development in non-

specific autoantibody-positive arthralgia, but did not perform multivariable analysis including all three autoantibodies with an autoantibody-negative group as reference[6]. In our study, anti-CarP antibodies were not independently associated with arthritis development and a significant effect of anti-CarP, additive to ACPA and RF, could not be shown. However, the current anti-CarP antibody test is not commercially available which allows further optimization and afterwards evaluation in larger studies.

ACPA- and RF-levels were rather stable over time, both in CSA-patients that developed clinical arthritis and in patients that did not progress. Seroconversion during the study was rarely observed for RF and absent for ACPA. The finding of stable ACPA-levels in the phase of CSA and during progression to clinical (persistent) arthritis suggests that the broadening of the autoantibody response may already have occurred in an earlier, and perhaps asymptomatic, pre-arthritis phase.

A large limitation of this study is the sample size of subgroup analyses; especially the ACPA-positive/RF-negative subgroup was small. Validation of the presented findings in other cohorts of arthralgia suspicious for progression to RA is needed. The primary outcome used was clinically apparent arthritis. As arthritis can be subtle in early stages and variation in the sensitivity to detect clinical arthritis between rheumatologists exists, sub-analyses were performed with clinical arthritis that was persistent at two subsequent visits or that was treated with DMARDs as outcome (both outcomes reflect chronic disease). These analyses provided similar results.

Based on results of case-control studies, revealing that simultaneous presence of ACPA and RF almost does not occur in healthy controls[20,21], it is sometimes suggested that presence of both ACPA and RF in arthralgia is a guarantee for progression to clinical arthritis and RA. However, this was not observed in the present study and our findings are in line with

results of other longitudinal studies. Bos et al[5] showed that 60% of ACPA-positive/RF-positive patients with non-specific arthralgia did not progress to clinical arthritis. Another study showed that 42% of ACPA-positive/anti-CarP-positive patients did not progress to arthritis[6]. Thus, previous studies also showed that presence of several autoantibodies in arthralgia was not always associated with arthritis development.

We hypothesized that ACPA-positive/RF-positive patients that did or did not progress would have lower autoantibody levels or less severe subclinical inflammation. However, no apparent differences were observed. An explanation for patients not progressing to clinical arthritis might be that the remaining ACPA-positive/RF-positive CSA patients will progress to clinical arthritis later on. Although we cannot exclude this, the Kaplan-Meier curves indicate that ACPA-positive/RF-positive patients mostly progressed in the first year and few converted in the second year. This makes the hypothesis that many subjects will progress after additional follow-up less likely. Other explanations are that patients that are truly pre-RA have differences in molecular characteristics of the autoantibodies themselves, or that another trigger (on top of the presence of autoantibodies) is required to develop clinically evident arthritis. This is a subject of further research.

In summary, presence of autoantibodies in CSA conferred increased absolute risks to develop clinical arthritis. Furthermore, positive predictive values in CSA were higher than that reported in non-specific arthralgia. However, also within CSA, presence of ACPA alone or a combination of ACPA and RF is insufficient to identify patients with imminent ACPA-positive RA with high accuracy (e.g. with PPVs >80%). Thus, in addition to clinical characteristics and autoantibodies, other biomarkers are needed for optimal prognostication.

Supporting information

Supplementary data is available at the website of Rheumatology (Oxford), or can be obtained by contacting the first author.

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Table 1. Baseline characteristics of the CSA-patients (N=241).

Patient characteristic		
Age in years, mean (SD)	44	(13)
Female sex, n (%)	187	(78)
Caucasian, n (%)	224	(93)
Family history of RA, n (%)	71	(30)
Symptom duration in weeks, median (IQR)	18	(10 – 48)
Presence of morning stiffness ≥60 minutes, n (%)	80	(33)
Current smoker, n (%)	54	(22)
BMI in kg/m^2, median (IQR)	26	(24 – 30)
Baseline HAQ-score, median (IQR)	0.50	(0.20 – 0.88)
68-TJC, median (IQR)	6	(3 – 10)
Increased CRP (>10 mg/L), n (%)	53	(22)
Positive for EULAR-definition for arthralgia suspicious for progression to rheumatoid arthritis[9], n (%)	178	(74)
Autoantibody status		
IgM-RF-positive (>3.5 IU/mL), n (%)	51	(21)
ACPA-positive (>7 U/mL), n (%)	32	(13)
Anti-CarP positive (>2 SD), n (%)	23	(10)

Legend:
ACPA = anti-citrullinated peptide antibody; BMI = body mass index; CRP = C-reactive protein; HAQ = Health Assessment Questionnaire; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; TJC = tender joint count.

Table 2. Test characteristics for Anti-Citrullinated Protein antibodies, Rheumatoid Factor and Anti-Carbamylated protein antibodies and conversion to clinical arthritis within two years as outcome (N=144).

	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	LR + (95%CI)	LR – (95%CI)
Evaluating antibodies individually						
ACPA +	50% (32%–68%)	92% (85%–96%)	63% (41%–80%)	88% (80%–93%)	6.3 (3.1–13.0)	0.54 (0.38–0.78)
IgM-RF +	60% (41%–77%)	86% (78%–92%)	53% (35%–70%)	89% (81%–94%)	4.3 (2.5–7.3)	0.47 (0.30–0.72)
Anti-CarP +	24% (11%–44%)	94% (87%–97%)	50% (24%–76%)	82% (74%–88%)	3.8 (1.4–9.9)	0.81 (0.66–1.0)
Evaluating combinations of ACPA and RF (ACPA- RF- as reference)						
ACPA + IgM-RF +	57% (34%–77%)	94% (87%–98%)	67% (41%–86%)	91% (84%–96%)	9.6 (4.1–22.7)	0.46 (0.28–0.75)
ACPA+ IgM-RF –	25% (6.7%–57%)	97% (91%–99%)	50% (14%–86%)	91% (84%–96%)	8.2 (1.9–36.0)	0.77 (0.56–1.1)
ACPA – IgM-RF +	40% (17%–67%)	90% (83%–95%)	38% (16%–64%)	91% (84%–96%)	4.2 (1.8–9.9)	0.66 (0.44–1.0)

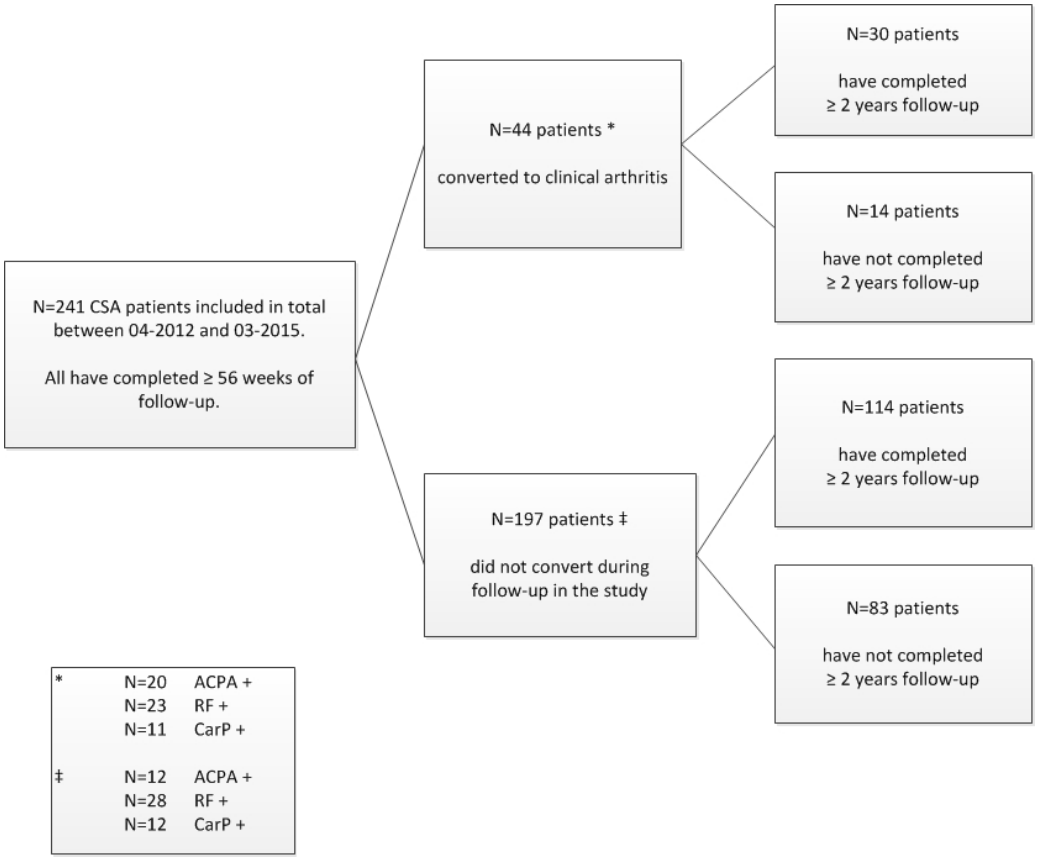
Legend:
IgM-RF = IgM rheumatoid factor; ACPA = anti-citrullinated protein antibodies; CarP = anti-carbamylated protein antibodies; 95%CI = 95% confidence interval; PPV = positive predictive value; NPV = negative predictive value; LR + = positive likelihood ratio; LR – = negative likelihood ratio.

Table 3. Baseline characteristics of ACPA-positive/Rheumatoid Factor-positive patients that or did not progress to RA to RA during 2 year follow-up.

Patient characteristic	Convertors (N=12)	Non-convertors (N=6)	p-value
Age in years, mean (SD)	45 (15)	49.8 (11)	0.50
Female sex, n (%)	9 / 12 (75)	5 / 6 (83)	0.69
Family history of RA, n (%)	4 / 12 (33)	0 / 6 (0)	0.11
68-TJC, median (IQR)	5.0 (3–8)	5.5 (2–9)	0.87
CRP			
Elevated CRP (>10 mg/L), n (%)	5 / 12 (42)	2 / 6 (33)	0.73
CRP-level, median (IQR)	4.7 (3–12)	4.2 (3–6)	0.32
IgM-RF level (IU/mL), mean (SD)	76.6 (77)	79.2 (64)	0.93
ACPA-level (U/mL), mean (SD)	222.8 (125)	211.0 (141)	0.87
MRI positive for inflammation			
Any inflammation present, n (%)	9 / 10 (90)	5 / 6 (83)	0.70
Total RAMRIS score, median (IQR)	5.8 (3–19)	5.8 (4–11)	0.36

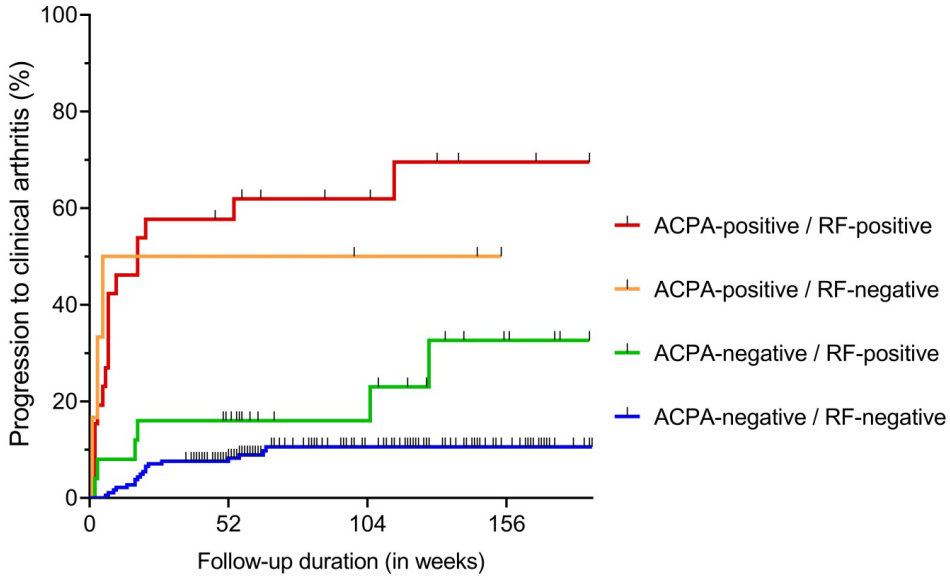
Legend:
ACPA = anti-citrullinated peptide antibody; CRP = c-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; MRI = magnetic resonance imaging; RA = rheumatoid arthritis; RAMRIS = Rheumatoid Arthritis MRI scoring system; SD = standard deviation; TJC = tender joint count. Symptoms were noted by rheumatologists as reported by the patients.

Figure 1. Flowchart of the patient flow and development of clinical arthritis during two-year follow-up period of the present study.



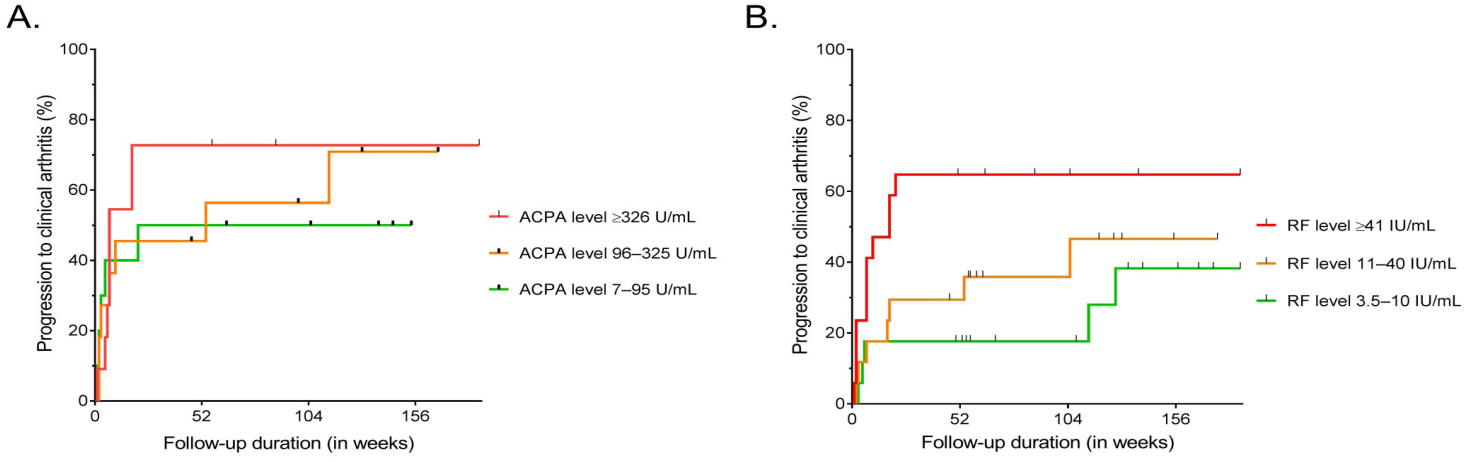
Legend:
Flowchart of the patient flow and development of clinical arthritis during two-year follow-up period of the present study.

Figure 2. Kaplan-Meier One Minus Survival plots with combinations of Anti-Citrullinated Protein antibodies (ACPA) and Rheumatoid Factor (RF) and associated risks for progression to clinical arthritis over time.



Legend:
With autoantibody-negative CSA-patients as reference (N=184), ACPA-negative/ RF-positive patients (N=25) had a HR of 2.6 (1.04-6.6), ACPA-positive/RF-negative (N=6) a HR of 8.0 (2.4-27.4) and ACPA-positive/RF-positive patients (N=26) a HR of 10.5 (5.4-20.6) for progression to clinical arthritis.

Figure 3. Kaplan-Meier One Minus Survival plots with (A) ACPA-levels in ACPA-positive CSA patients and (B) RF-levels in RF-positive CSA-patients and associated risks for progression to clinical arthritis over time.



Legend:

A. The ACPA-positive patients in the second tertile (levels 96–325 U/ml, N=11) had a HR of 1.2 for progression to clinical arthritis (95%CI=0.39–3.9) compared to the patients in the lowest tertile (N=10). The ACPA-positive patients in the third and highest tertile (levels ≥326 U/ml, N=11) had a HR of 1.6 for progression to clinical arthritis (95%CI=0.50–4.8) compared to the patients in the lowest ACPA-level tertile.

B. The RF-positive patients in the second tertile (levels 11-40 IU/ml, N=17) had a HR of 1.6 for progression to clinical arthritis (95%CI=0.50–5.0) compared to the patients in the lowest tertile (N=17). The RF-positive patients in the third and highest tertile (levels ≥41 IU/ml, N=17) had a HR of 3.3 for progression to clinical arthritis (95%CI=1.1–9.6) compared to the patients in the lowest RF-level tertile.

Chapter 4

Screening for two or three autoantibodies in persons at risk for RA – implications of current data for clinical practice

R.M. ten Brinck [1], L.A. Trouw [2], Annette H.M. van der Helm–van Mil [1,3]

1. Department of Rheumatology, Leiden University Medical Center, Leiden, the Netherlands
2. Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Rheumatology, Erasmus Medical Centre, Rotterdam, The Netherlands

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Sir,

In recent years, research has focused on identifying novel autoantibodies and their value for early identification of rheumatoid arthritis (RA). A frequently studied novel autoantibody is anti-carbamylated protein (anti-CarP)[1,2] Presence of this autoantibody at diagnosis of RA is associated with a higher disease activity and a more destructive disease course.[3] The literature on the value of this autoantibody, in addition to the evaluation of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF), in persons at risk for RA was recently meta-analyzed.[4] It was demonstrated that combined presence of ACPA, RF and anti-CarP rarely occurred in control groups and therefore was highly specific for RA. Although promising, three issues need to be considered before it can be concluded that assessing three autoantibodies in the same individuals is more beneficial than evaluation of ACPA and RF only.

First, current summarized data were retrieved from case-control or nested case-control studies. Caution is advised when utilizing case-control studies for assessing diagnostic accuracy. Case-control studies comparing established patients with controls are typically used to screen the utility of a test and, if an effect in case-controls studies is observed, longitudinal studies in clinically relevant populations are subsequently needed to evaluate the diagnostic accuracy.[5] Effect sizes in this second step are often lower than observed in the first step: two- or three-fold lower effect sizes have been reported.[5,6] Especially studies comparing cases to healthy volunteers as controls harbor the risk of inflated estimates of diagnostic accuracy.[10] A second issue is what autoantibodies to test. Is presence of anti-CarP indeed of added value to ACPA and RF (that are already routinely determined in clinical practice)? Thirdly, the prognostic value may depend on the 'at risk population' and be different for persons without symptoms (e.g. first degree relatives) or patients seeking medical help because of symptoms. Observational longitudinal studies in several of these populations are crucial to decide if testing all three autoantibodies is of added value to identify patients with imminent RA.

To address these issue in patients that are considered to be in the symptomatic pre-arthritis phase, we performed additional analyses in our longitudinal cohort study on consecutive patients that presented with Clinically Suspect Arthralgia (CSA)[7] to evaluate the diagnostic accuracy of three positive autoantibodies for RA development.

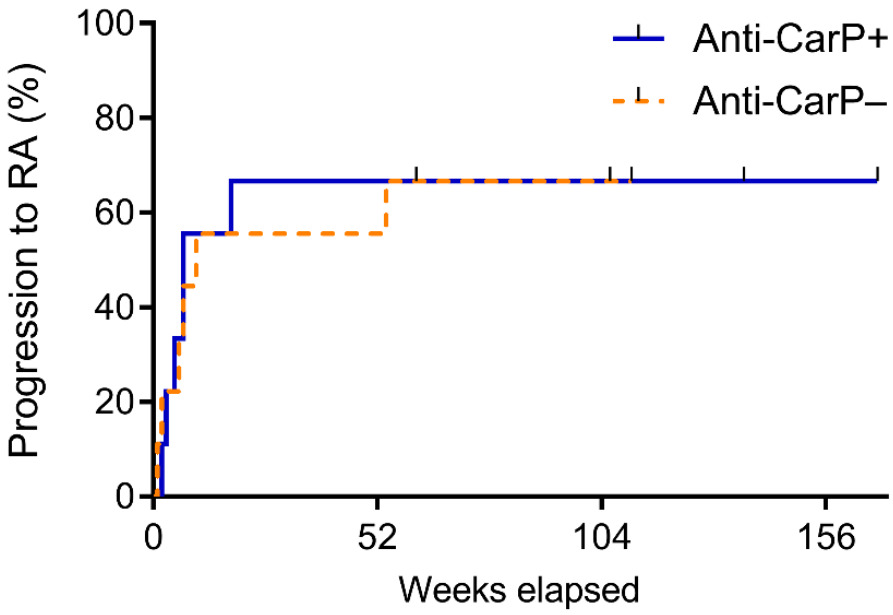
241 patients with CSA, as described previously[7], presented with arthralgia of small joints of recent onset, without clinical arthritis and were considered to be at risk for progression to RA by their rheumatologist based on the clinical presentation (autoantibody status was generally unknown).[8] After inclusion, IgG ACPA (EliA CCP (anti-CCP2), Phadia, Nieuwegein, the Netherlands; positive if ≥7 U/ml)), IgM RF (in-house ELISA; positive if ≥3.5 IU/ml) and IgG anti-CarP antibodies (in house ELISA based on the use of carbamylated fetal calf serum, and non-modified fetal calf serum as the coating antigens as a control as described previously[2]; cut-off equivalent to 2 S.D. above the mean in a group of healthy controls) were determined. The outcome was clinical arthritis and diagnosis of RA after 1-year follow-up.

With autoantibody-negative patients as reference (n=184), specificity of presence of all three autoantibodies was high: 97% (95% confidence interval: 93–100%) with a sensitivity of 46% (19–73%). The positive predictive value (PPV) was 67% (36–97%), negative predictive value 93% (88–98%), the positive likelihood ratio (LR) 14 (4.0–50) and negative LR 0.56 (0.34–0.92). These results confirm the high specificity of three autoantibodies. The PPV of ACPA+RF+anti-CarP+ in this group (n=9) was identical to ACPA+RF+anti-CarP– (also 67% (36–97%), n=9).[7] Thus the PPV did neither increase nor decrease after adding anti-CarP. In Cox proportional hazards regression analyses, the added value of Anti-CarP as a third autoantibody in ACPA+/RF+ patients revealed a hazard ratio for Anti-CarP+ of 1.03 (95%CI 0.33–3.2; p=0.97; Figure 1), indicating no increased risk increase risk for RA in patients with three positive autoantibodies compared to ACPA- and RF-positive persons.

Based on these data, the added value of testing anti-CarP as a third autoantibody in arthralgia patients presenting at secondary care would seem limited. Importantly, these data cannot be extrapolated to the setting of populations without symptoms. Future population-based longitudinal studies will have to demonstrate if a combination of three autoantibodies can contribute to the identification of at-risk patients in the stage preceding the onset of arthralgia.

In conclusion, in follow-up to results from well-conducted case controls studies, the first longitudinal data obtained in a clinically relevant population of arthralgia patients illustrate the phenomenon that case-control studies may result in inflated effect sizes of diagnostic accuracy. At present, evidence is lacking to measure a third autoantibody on top of ACPA and RF in patients presenting with arthralgia in clinical practice. Our data should be interpreted in the context of the limitation of small absolute numbers, and more research in larger longitudinal studies is needed.

Figure 1. Kaplan-Meier plot presenting no difference in progression to RA in patients with Clinically Suspect Arthralgia



Legend:
With CSA-patients that were positive for ACPA and RF, but not harbouring Anti-CarP antibodies as reference, ACPA+/RF+ patients that also had Anti-CarP had no higher risk for development of clinical arthritis: hazard ratio 1.03 (95%CI 0.33–3.2; p=0.97).

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Chapter 5

Functional limitations in the phase of Clinically Suspect Arthralgia are as serious as in early clinical arthritis; a longitudinal study

R.M. ten Brinck [1], H.W. van Steenbergen [1], L. Mangnus [1], L.E. Burgers [1], M. Reijnierse[2], T.W.J. Huizinga [1], A.H.M. van der Helm–van Mil [1]

1. Department of Rheumatology, Leiden University Medical Center, Leiden, the Netherlands.
2. Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands.

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Abstract

Introduction: A phase of arthralgia may precede the emergence of rheumatoid arthritis (RA). Although several studies have focussed on biomarkers, the relevance of this phase for patients is less studied. It is unknown if patients already have functional limitations and if this is correlated to the extent of subclinical inflammation. Therefore we assessed functional disability in patients with Clinically Suspect Arthralgia (CSA), its association with MRI-detected subclinical inflammation, and its course during progression to clinical arthritis.

Methods: From April 2012-March 2015, 241 patients had arthralgia for <1 year and were, based on clinical presentation, considered at risk for RA by their rheumatologists. At baseline, Health Assessment Questionnaire (HAQ)-scores were determined and unilateral 1.5T MRI of MCP, wrist and MTP-joints made. The extent of MRI-detected subclinical inflammation was assessed by summing the synovitis, tenosynovitis and bone marrow edema scores (range 0-189). Patients were followed on arthritis development and HAQ-scores were repeated when clinical arthritis had developed.

Results: The median HAQ-score at presentation with CSA was 0.50. Higher MRI-inflammation scores were associated with higher HAQ-scores (β=0.017, 95%CI=0.004-0.030). During median 103 weeks follow-up, 44 patients progressed to clinical arthritis. HAQ-scores ≥1.0 were associated with arthritis development (HR=2.50, 95%CI=1.03-6.10). Within converters, median HAQ-scores did not increase from presentation with CSA to arthritis development (0.88 and 0.75, p-value=0.36).

Conclusions: HAQ-scores ≥1.0 at presentation associated with the development of clinical arthritis. Functional limitations in the pre-arthritis phase of CSA were as serious as in the early clinical phase, demonstrating the relevance of CSA from patients' perspectives.

Introduction

Within rheumatoid arthritis (RA) a symptomatic phase may precede the development of clinical arthritis[1]. A broad range of symptoms and signs has been described in this phase[2]. In addition, it has been established that presence of autoantibodies[3,4], increased levels of acute phase reactants[5] and Magnetic Resonance Imaging (MRI)-detected subclinical inflammation[6] are associated with progression to clinical arthritis. Although several biomarkers have been studied, it is still unknown to what extent patients with arthralgia at risk for RA experience functional disability. In addition, it is undetermined if functional disability in this disease stage is associated with subclinical inflammation and if the functional disability increases during progression to clinical arthritis.

The Health Assessment Questionnaire (HAQ) is a commonly used instrument to measure self-reported functional disability in patient groups[7]. From the general population it is known that HAQ-scores increase with age[8] and are higher for women[9]. The median HAQ-score for patients presenting with RA is generally 1.0[9,10]. It has been demonstrated that MRI-detected inflammation in RA was associated with increased functional impairment at 6-years follow-up[11].

In order to increase the comprehension of patients' experiences on physical functioning in a symptomatic pre-arthritis stage, this study evaluated patients without clinical arthritis but with arthralgia that were considered at risk for progression to RA by their rheumatologists (Clinically Suspect Arthralgia). This study assessed 1) the level of functional disability measured with HAQ-scores in CSA, 2) the association of functional disability with the severity of MRI-detected subclinical inflammation, 3) the association of functional disability with progression to clinical arthritis and 4) the course of HAQ-scores during progression from CSA to clinical arthritis.

Methods

Patients

Two hundred and forty-one patients were consecutively included between April 2012-March 2015 in the Leiden Clinically Suspect Arthralgia (CSA) cohort. CSA-patients had recent-onset (<1 year) arthralgia of hand or feet joints and were considered at risk for RA based on the clinical expertise of the rheumatologists[6]. Per definition CSA was not present if patients presented with clinical arthritis or if another explanation for the symptoms (e.g. osteoarthritis or fibromyalgia) was more likely than imminent RA. Hence, as described previously[6], inclusion was mainly based on clinical expertise and patients with evident other diagnoses were not studied. Furthermore, laboratory results were largely unknown at first visit as general practitioners were discouraged to perform additional tests, hence inclusion in the cohort was largely based on the findings obtained at history taking and physical examination. At baseline, questionnaires were completed, among which HAQ and Visual Analogue Scale (VAS, range 0–10) for pain. Within two weeks after inclusion, an MRI was performed. The design of the cohort is further described in reference 6. Baseline HAQ-scores were missing in 37 patients (15.4%). No differences were found in baseline characteristics for the patients with known and unknown HAQ-scores (Additional File 2).

Health Assessment Questionnaire

The Health Assessment Questionnaire Disability Index was used. It comprises 20 questions, covering eight categories: dressing and grooming, arising, eating, walking, hygiene, reach, grip and activities of daily living. Scores for each category consist of a scale, ranging 0–3, with 0 being no disability and 3 representing full disability. The scoring system is based on the highest abnormal response in each category. The total HAQ-score consists of an average of the eight categories.

Subclinical inflammation detected by Magnetic Resonance Imaging

Unilateral contrast-enhanced MRIs were made of the 2nd-5th metacarpophalangeal, wrist and 1st-5th metatarsophalangeal joints of the most painful side, or the dominant side in case of equally severe symptoms at both sides. Patients were instructed not to use NSAIDs 24 hours prior to MRI. An MSK-extremity 1.5 Tesla MRI-scanner was used. The detailed MR protocol is provided in Additional File 1. In short, before contrast-enhancement a T1-weighted sequence was acquired of MCP and wrist joints in the coronal plane. Postcontrast, T1-weighted, fat saturated sequences were acquired in coronal and axial planes. The foot was scanned with two protocols. In the first 78 patients a T1-weighted sequence and a T2-weighted fat saturated sequence were acquired in the axial plane (relative to the anatomical position), before contrast agent administration. In the remaining 163 patients postcontrast, T1-weighted, fat saturated sequences were acquired in axial and coronal planes. This provided more information while reducing scanning-times. MRIs were scored for bone marrow edema (BME) and synovitis as defined by the OMERACT Rheumatoid Arthritis MRI Scoring system (RAMRIS)[12]. Tenosynovitis was scored as described by Haavardsholm and colleagues (also applied at flexor and extensor tendons of 2nd-5th MCP-joints)[13]. The sum of scores for synovitis, tenosynovitis and BME yielded the total MRI-inflammation score; the total score ranged between 0-189. Scoring was performed by two independent trained readers (HWvS, LM) blinded to clinical data. Within-reader intraclass correlation coefficients (ICC) for the total MRI-inflammation score were 0.98 and 0.99; between-reader interclass correlation coefficient was 0.96. Mean scores of the two readers were used in analyses.

Follow-up

Scheduled follow-up visits were performed at 4,12 and 24 months. Additional visits took place at indication; either if preferred by the patient (because of an increase in symptom) or if felt necessary by the

rheumatologist). The patients included in this study were all followed for development of clinically apparent arthritis for ≥1 year. Medical files were studied for established arthritis until April 22nd 2016. Patients were not treated with disease-modifying anti-rheumatic drugs (including steroids) in the phase of CSA; NSAIDs were allowed. Time to clinical arthritis was defined as time from inclusion in the cohort to the date of first detection of clinical arthritis. Patients who did not develop arthritis were censored at the date that all medical files were studied on arthritis development or at the last follow-up visit. If patients developed clinical arthritis, the HAQ was repeated at that visit.

Statistical analyses

Univariable linear regression models were used to investigate the association between subclinical MRI-inflammation and HAQ-scores; models were adjusted for age at inclusion. Univariable Cox proportional hazards regression analyses were used to calculate hazard ratios for HAQ-scores in relation to arthritis development. Patients were appointed into quartiles according to their total HAQ-score to create four subgroups with equal numbers. Cox regression was repeated with development of RA according to the 2010 classification criteria[14] as outcome. In multivariable Cox regression the analysis was adjusted for age, gender, presence of MRI-subclinical inflammation and ACPA-status. A paired t-test was performed to compare HAQ-scores and VAS-scores for pain at presentation with CSA and after conversion to clinical arthritis; patients that completed the HAQ or VAS-score ≥1 week after DMARD-initiation were excluded from these analyses. SPSS, version 23.0 was used. P-values <0.05 were considered significant.

Results

CSA-patients

Baseline characteristics of the 241 patients included are shown in Table 1. The mean age was 44.3 years, 78% were female. The median HAQ-score of the total group of patients at baseline was 0.50 (interquartile range: 0.25–0.88).

HAQ-score and MRI-detected subclinical inflammation at presentation

The association between severity of MRI-detected subclinical inflammation and functional disability was corrected for age. CSA-patients that presented with higher total MRI-inflammation scores had higher HAQ-scores (β=0.017, 95%CI=0.004-0.030, p=0.010). This β indicates that per point increase in MRI-inflammation score, the HAQ-score increased with 0.017 (for interpretation the MRI-inflammation score ranges between 0-189). The synovitis, tenosynovitis and bone marrow edema scores were also studied separately. Of the individual types of inflammation, the tenosynovitis score showed the strongest association with functional disability (β=0.046, 95%CI=0.017–0.076), versus β=0.024 (95%CI=-0.008–0.057) and β=0.026 (95%CI=-0.004–0.057) for synovitis and bone marrow edema respectively.

HAQ-scores at presentation and progression to clinical arthritis

During a median follow-up period of 103 weeks, 44 patients progressed to clinical arthritis. The patients that progressed presented with higher baseline HAQ-scores than CSA-patients that did not progress (median 0.88 versus 0.50). Four subgroups with equal numbers were created to study the association between HAQ-scores and progression to clinical arthritis in more detail (the quartiles contained four groups with HAQ-scores of <0.25, 0.25–0.50, 0.63–0.88 and ≥1.0, Additional File 3). Patients with HAQ-scores ≥1.0 had a significantly increased hazard on developing clinical arthritis (HR=2.50, 95%CI=1.03–6.10), compared to the patients with HAQ-scores <0.25 (Figure 1).

Multivariable Cox regression was performed to investigate the association between HAQ-scores and arthritis development, adjusting for age, gender, ACPA-status and presence of MRI-detected subclinical inflammation. Higher HAQ-scores remained significantly associated with arthritis development with HAQ-scores <0.25 as reference: HR 2.6 (95%CI=1.05–6.6) for HAQ-scores ≥1.0. The presence of a positive ACPA-test and the presence of MRI-detected subclinical inflammation were also significantly associated with arthritis development in this model (HR=6.7, 95%CI=3.4–13.8 and HR=3.3, 95%CI=1.4–8.0 respectively). No significant associations were observed for age (HR=0.98, 95%CI=0.95–1.01) or gender (HR=0.88, 95%CI=0.40–2.0).

27 of the patients with arthritis fulfilled the 2010-criteria for RA[14] already at the first visit with clinical arthritis. Additional File 4 provides the results of sub-analyses with RA as outcome. Similar findings were obtained with the highest HR for the group of patients with a HAQ>1.0 (HR 2.7, 95%CI 0.85–8.5).

HAQ and VAS for pain at presentation with CSA and at arthritis development

HAQ-scores at clinical presentation with CSA and after arthritis development (but before DMARD-initiation) were available in 25 patients. On group level, median HAQ-scores did not differ between the time of presentation with CSA and the time of presentation with clinically apparent arthritis: median 0.88 (IQR 0.38–1.2) versus 0.75 (IQR 0.38–1.3) respectively, p-value=0.36 (Figure 2). Intra-individual changes in HAQ-scores over time are shown in Additional File 5. Also in the subgroup of patients that fulfilled the 2010-criteria for RA (n=21), the median HAQ did not increase between presentation with CSA and clinical arthritis development (0.82 and 0.75, p=0.47).

The progression from CSA to clinical arthritis is based on an increase in local joint inflammation. As functional limitations may not only associate with inflammation, but also be a direct consequence of pain, we also explored the overall level of pain (measured on a VAS ranging 0–10) in CSA and at conversion to clinical arthritis. The VAS-score for pain showed a non-significant tendency towards an increase between the phase of CSA and that of early clinical arthritis (median 6.0 and 7.0 respectively, p-value=0.11, Figure 2). Thus despite an increase in inflammation and pain, functional disability was already maximal in the phase of CSA.

Discussion

This longitudinal study showed that patients that develop clinical arthritis already have functional limitations in the phase of arthralgia. HAQ-scores at group level were similar at the time of presenting with CSA and after emergence of clinical arthritis. Furthermore, severity of MRI-detected subclinical inflammation is associated with the severity of functional impairments. Together, these data demonstrate the functional relevance of the HAQ and MRI-detected subclinical inflammation in symptomatic patients in the pre-arthritis stage. This suggests that, although occurrence of clinically detectable arthritis is a major event from the rheumatologist's perspective (as this is mostly the moment of initiation of DMARD-therapy), it is of less importance for patients from a functional perspective.

CSA-patients with HAQ-scores ≥1.0 in particular were at increased risk of progression to clinical arthritis. Interestingly, previous studies in early RA cohorts have shown that mean HAQ-scores at presentation were 1.0[9,10]. This suggests that functional impairments in the symptomatic pre-arthritis and early clinical phases are of similar severity. A presumption that is further supported by our findings that the CSA-patients that progressed

to clinical arthritis did not experience an increase in functional disability, in other words the maximal level of disability was already present when presenting with CSA.

Patients that presented with CSA but did not progress to clinical arthritis presumably also had more functional impairments than the general population, as their median HAQ was 0.50 and mean HAQ-score of an age-related normal population (women aged 40-44 years) is approximately 0.08[8].

We observed that a HAQ ≥1.0 was associated with progression to clinical arthritis, independent of other predictors (age, gender, ACPA, MRI-detected inflammation). Though this study was not aimed at identifying novel markers for progression from CSA to RA, but to explore the level of functional disability in patients with CSA and during progression to clinical arthritis. The question if a HAQ-score is valuable for diagnostic or prognostic purposes needs to be studied in further, larger studies.

The severity of functional disability was associated with the severity of MRI-detected subclinical inflammation, indicating that the functional impairments were in part related to (subclinical) inflammation. Previous studies in early arthritis or in RA also showed an association between MRI-detected inflammation and HAQ-scores within RA[11,15]. In one of these studies it was observed that MRI-detected tenosynovitis had the strongest association with functional disability in early arthritis.[15] Interestingly, also in patients with CSA we observed that tenosynovitis had the strongest association with functional disability. The beta of MRI-detected tenosynovitis of 0.046 indicates that a MRI-inflammation score of 6 associated with an increase in HAQ of 0.27. Although statistically significant, the relatively small effect size indicates that the functional disability in CSA is only partly explained by (MRI-detected) subclinical inflammation.

A potential weakness is that 37 patients did not complete the baseline HAQ. Because the baseline characteristics of the patients that had HAQ-data and those without HAQ-data were similar, we believe that there is no important bias.

Patients were not included if the treating rheumatologist considered another explanation for the arthralgia (e.g. osteoarthritis or fibromyalgia) more likely than imminent RA. Therefore, we think it is unlikely that patients with forms of (chronic) pain syndromes might have skewed the data towards higher HAQ-scores. Furthermore, we use medians for comparisons of HAQ- and VAS-scores as these are more resistant against outliers.

Another potential limitation for the analyses is that both HAQ-scores and MRI-inflammation scores are assessed at semi-quantitative scales. However, the HAQ is one of the most important and validated patient-reported outcomes in RA.

Finally, it should be taken into consideration that the sample size of patients converting to clinical arthritis is relatively small. Our study is nevertheless the largest to date to investigate functional disability in patients with clinically suspect arthralgia.

Ideally, to fully evaluate the burden of clinically suspect arthralgia on functional disability, the functional status of the patients with CSA included in this study should be compared to age- and sex-matched controls from the general population. As such references were not available for the Dutch population, we could not perform such comparison.

In conclusion, functional disabilities exist already in the symptomatic pre-arthritis phase, with (on group level) a similar severity as when presenting with clinical arthritis. Although occurrence of clinically detectable arthritis

is a major event from the rheumatologist's perspective (as then initiation of DMARD-therapy is warranted), the present data illustrate the importance of the symptomatic pre-arthritis phase from a functional perspective.

Supporting information

Supplementary data is available at the website of RMD Open, or can be obtained by contacting the first author.

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Table 1. Patient characteristics (N=241) at baseline presentation with CSA.

Patient characteristic		
Age in years, mean (SD)	44.3	(12.9)
Female sex, n (%)	187	(77.6)
Family history of RA, n (%)	71	(29.5)
Symptom duration in weeks, median (IQR)	18.4	(9.7–48.2)
Presence of morning stiffness ≥60 minutes * ‡, n (%)	80	(33.2)
BMI in kg/m^2, median (IQR)	26.1	(23.6–29.9)
68-TJC, median * (IQR)	6	(3–10)
Current smoker, n (%)	54	(22.4)
Autoantibody status		
ACPA-positive (>7 U/mL), n (%)	32	(13.3)
IgM-RF-positive (>3.5 IU/mL), n (%)	51	(21.2)
Increased CRP (>10 mg/L), n (%)	53	(22.0)
Baseline VAS pain score, median (IQR)	5	(3–7)
Baseline HAQ-score *		
First quartile (N=44)	<0.25	
Second quartile (N=62)	0.25 – 0.50	
Third quartile (N=51)	0.63 –0.88	
Fourth quartile (N=47)	≥1.0	

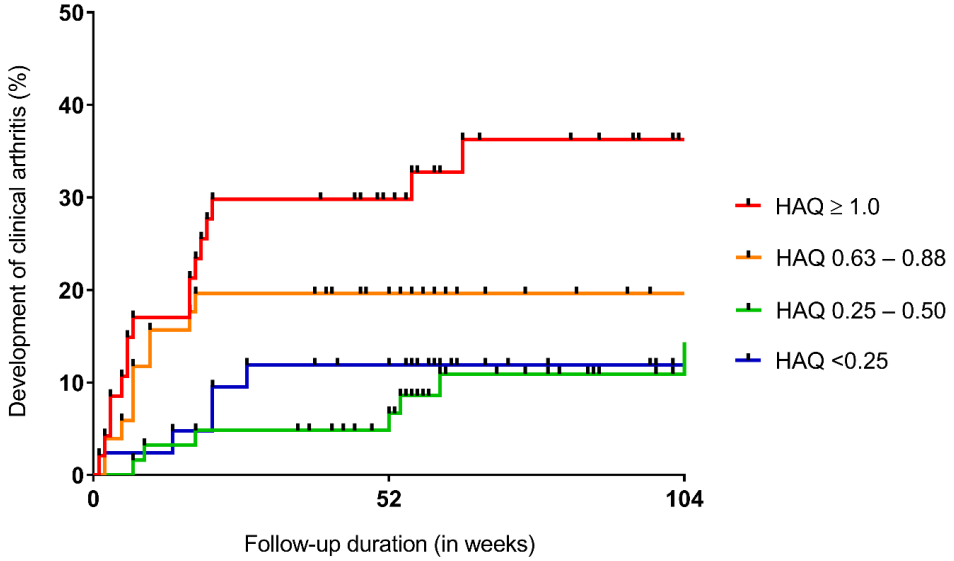
Symptoms were noted by rheumatologists as reported by the patients.

* Missing data were as follows: Morning stiffness (27), 68-TJC (4), HAQ-score (37).

‡ The presence of symptoms refers to the presence of symptoms at the baseline visit.

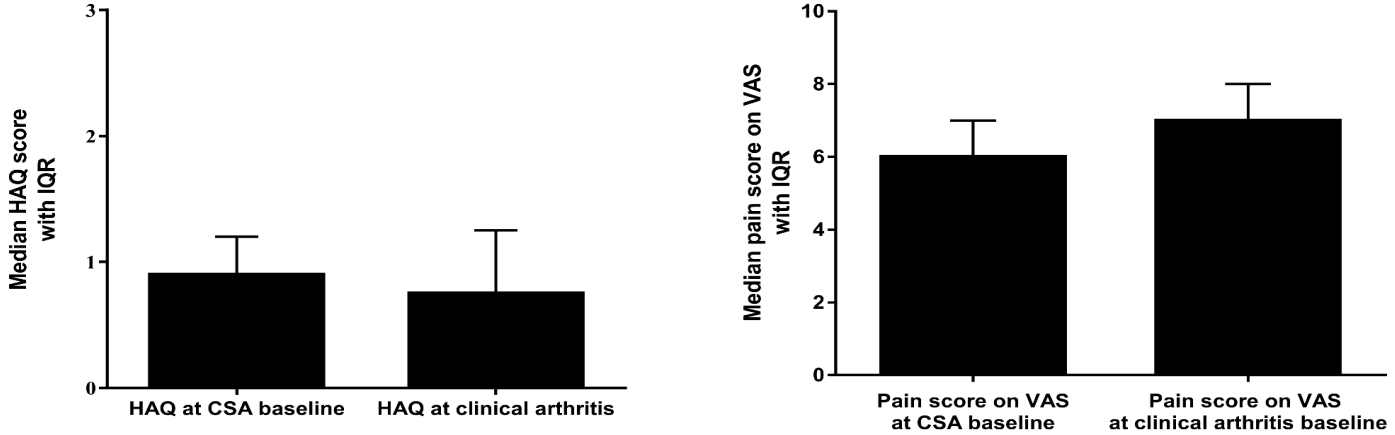
Legend:
ACPA = anti-citrullinated peptide antibody; BMI = body mass index; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; HAQ = Health Assessment Questionnaire; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; TJC = tender joint count.

Figure 1. Kaplan-Meier One Minus Survival plot showing cumulative progression to clinical arthritis for CSA-patients divided in four groups based on their baseline HAQ-score.



Legend:
Patients were appointed into quartiles according to their total HAQ-score to create four subgroups with equal numbers, see Additional File 3. Each line represents one HAQ-score quartile and cumulative progression to clinical arthritis. The lowest quartile contains patients with HAQ-scores <0.25 with N=44 was the reference group. The second quartile contains HAQ-scores 0.25–0.50 (N=62), with a HR for progression to clinical arthritis of 0.67 (95%CI=0.24–1.9). Patients in the third quartile had HAQ-scores 0.63–0.88 (N=51) with a HR for progression to clinical arthritis of 1.3 (95%CI 0.49–3.4). Finally, the quartile with the highest HAQ-scores contains HAQ-scores ≥ 1.0. (N=47).The hazard ratio for this quartile (HR=2.50, 95%CI=1.03–6.10) was significantly elevated, compared to the lowest quartile.

Figure 2. Column bar graphs showing HAQ-scores and pain score on VAS (scale 0-10) that were collected at presentation with CSA and after progression to clinical arthritis but before DMARD initiation.



Legend:
Column bar graphs showing HAQ-scores and pain score on VAS (scale 0-10) of 25 patients that were serially collected at presentation with CSA (left bar) and after progression to clinical arthritis but before DMARD initiation (right bar). A paired t-test for comparisons of median HAQ-scores and pain scores on VAS (scale 0-10) at both time points (CSA baseline inclusion and the moment of established arthritis) revealed no differences (p-value=0.36 and p=0.11, respectively).

Part II

Disease mechanisms involved in progression from CSA to RA

Chapter 6

Inflammation functions as a key mediator in the link between ACPA and erosion development: an association study in Clinically Suspect Arthralgia

R.M. ten Brinck [1], R.E.M. Toes [1], A.H.M. van der Helm–van Mil [1,2]

1. Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
2. Department of Rheumatology, Erasmus Medical Center, Rotterdam, The Netherlands

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Abstract

Background: Anti-citrullinated protein antibodies (ACPA) associate with more severe joint erosions in rheumatoid arthritis (RA), but the underlying mechanism is unclear. Recent in vitro and murine studies indicate that ACPAs can directly activate osteoclasts leading to bone erosions and pain. This study sought evidence for this hypothesis in humans and evaluated in arthralgia patients at risk for RA whether ACPA associated with erosions (detected by MRI) independent of inflammation, and also independent of the presence of Rheumatoid Factor (RF).

Methods: 507 patients with Clinically Suspect Arthralgia underwent ACPA- and RF-determination and 1.5T contrast-enhanced MRI of metacarpophalangeal, wrist and metatarsophalangeal joints at baseline. MRIs were scored for presence of local inflammation and erosions. Comparisons of erosion scores were performed using Kruskal-Wallis tests. To evaluate if inflammation is, in statistical terms, intermediary in the causal path of ACPA and erosions, three-step mediation analysis was performed using linear regression.

Results: ACPA-positive patients had higher erosion scores than ACPA-negative patients ($p=0.006$). ACPA-positive patients without subclinical inflammation did not have higher erosion scores than ACPA-negative patients ($p=0.68$), in contrast to ACPA-positive patients with local inflammation ($p<0.001$). Mediation analyses suggested that local inflammation is in the causal path of ACPA leading to higher erosion scores. Compared to ACPA-negative/RF-negative patients, ACPA-positive/RF-negative patients did not differ ($p=0.30$), but ACPA-positive/RF-positive patients had higher erosion scores ($p=0.006$).

Conclusions: The effect of ACPA on erosions is mediated by inflammation and is not independent of RF.

Background

Although anti-citrullinated protein antibodies (ACPA) are the most important risk factor for joint destruction in rheumatoid arthritis (RA), the underlying pathophysiological process is unclear. Traditionally, it is hypothesized that ACPAs can enhance inflammation[1] (for instance via immune complexes that stimulate macrophages to secrete pro-inflammatory cytokines) and that inflammation is required for destruction, resulting in e.g. visible bone erosions on radiographs. Recent *in vitro* studies and mouse models have generated a new concept in which ACPA can directly induce osteoclast activation, followed by autocrine enhancement of osteoclast maturation and activation[2,3]. This may subsequently lead to bone loss (and pain) as observed in studies performed *in vivo* following injection of ACPA[2-5]. The findings that ACPA can be present long before synovitis is clinically detectable[6] and that sensitive imaging techniques have detected small erosions in arthralgia patients[7] fit with the hypothesis that joint inflammation is not necessary to generate erosions[5]. Despite observations made *in vitro* and *in vivo* in mice[2,3], there is presently little information available on data from ACPA-positive patients in the absence of local inflammation. Hence, it is not known if ACPA can lead to bone erosions only with concurrent presence of inflammation, or that ACPA induces direct osteoclast activation (leading to erosions without requiring concomitant inflammation) in humans as well. By performing association studies in patients that are in the disease phase of arthralgia without the presence of clinical synovitis, information on these relationships can be obtained as only part of the arthralgia patients display subclinical inflammation. Therefore, the arthralgia setting provides the possibility to study associations of ACPA, (local) inflammation and erosions.

Likewise, this setting can also answer whether the effect of ACPA –if any– is dependent on the presence of Rheumatoid Factor (RF). Studies within early (rheumatoid) arthritis, using high-resolution CT, have shown that combined presence of ACPA and RF is associated with the number and

size of erosions rather than ACPA individually[8]. In addition, it has been shown that early arthritis patients that harbour both ACPA and RF display increased osteitis scores as detected by Magnetic Resonance Imaging (MRI), in contrast to ACPA single-positive patients[9].

With the aim to find support that ACPAs themselves are directly linked to bone erosions in humans, this study in patients with Clinically Suspect Arthralgia evaluated whether 1) ACPA associated with higher erosion scores (detected by MRI) independent of the presence of inflammation, and 2) whether higher erosion scores were associated with ACPA alone or with ACPA and RF combined.

Patients and Methods

Patients

507 arthralgia patients consecutively included in the Leiden Clinically Suspect Arthralgia cohort between April 2012 and September 2017 were studied. Clinically Suspect Arthralgia was defined as: recent-onset (<1-year) arthralgia of small joints, without clinically detectable synovitis (i.e. joint swelling) at physical examination, while the treating rheumatologists considered the patients suspicious for progression to RA based on their clinical presentation[10]. General practitioners in our region rarely performed ACPA- or RF-testing before referral[11]; hence this infrequently affected inclusion decisions[10]. After inclusion, ACPA (EliA CCP (anti-CCP2), Phadia, Nieuwegein, the Netherlands; positive if ≥7 U/mL), RF (as described previously, in-house ELISA[12]; positive if ≥3.5 IU/mL) and C-Reactive protein (CRP; positive if ≥5.0 mg/L) were determined. The cohort is described in detail in reference [10]. Informed consent was obtained in all subjects. The local medical ethical committee approved the study.

Within 1-2 weeks after inclusion, 1.5T contrast-enhanced MRI was made of MCP2-5, wrist and MTP1-5 joints of the most painful side (see

Supplementary Methods for MRI protocol). Disease modifying anti-rheumatic drugs were not used, NSAIDs were stopped 24 hours before MRI. MRIs were scored for erosions, Bone Marrow Edema (BME), synovitis[13] and tenosynovitis[14] by two readers as described in the Supplementary Methods. Within-reader intraclass correlation coefficients (ICC) were 0.98 and 0.99; between-reader ICC was 0.96.

Inflammation

Inflammation was assessed in two ways: local inflammation was considered present if ≥1 joint had MR-detected BME, synovitis or tenosynovitis that was more than that observed in age-matched symptom-free controls[15] (Supplementary Methods). Secondly, 'any inflammation' was defined as the presence of either local subclinical inflammation (MRI-detected synovitis, BME or tenosynovitis) and/or an elevated C-Reactive Protein level. In this second analysis, 'any (i.e. systemic) inflammation' was taken into consideration as it could be argued that the presence of increased acute phase reactants in patients that have no subclinical joint inflammation as detected with MRI indicates that some inflammation is present in these patients.

Analyses

Comparisons of erosion scores were performed using Kruskal-Wallis tests. To evaluate if inflammation is, in statistical terms, intermediary in the causal path of ACPA and erosions, mediation analyses were performed as described by Baron and Kenny[16]. Here, linear regression was used to evaluate in three steps if local inflammation is a mediator in the causal path of ACPA presence and erosion score as outcome. First, the association between presence of ACPA and erosions was investigated. Second, the association between presence of ACPA and severity of local inflammation was investigated. Finally, both ACPA and local inflammation were entered and tested whether this effect was different from the association of ACPA alone and erosion score. The percentage of mediation was calculated. All

regression analyses were corrected for age. Additionally, a triple stratification was applied for ACPA, RF and local subclinical joint inflammation. Statistical Package for the Social Sciences (SPSS) version 23.0 was used.

Results

Patients with CSA had a mean age of 44 years, 77% were female and presence of local subclinical joint inflammation on MRI was observed in 50% (N=255). 64% of the patients included met the EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis (3/7 items present)[17]. Further characteristics are shown in Table 1.

ACPA with concomitant inflammation, but not ACPA alone, associated with higher erosion scores

First a comparison was made between all ACPA-positive patients and ACPA-negative patients: ACPA-positive patients had higher erosion scores than ACPA-negative patients ($p=0.006$; Figure 1A). Also the presence of MRI-detected subclinical inflammation was associated with higher erosion scores ($p<0.001$; Figure 1B).

Next, stratification was applied for both ACPA and local subclinical joint inflammation. After this stratification, it was observed that in the absence of local subclinical inflammation ACPA-positive (ACPA+/MRI–) patients did not have higher erosion scores than ACPA-negative (ACPA–MRI–) patients ($p=0.68$). In contrast, ACPA-positive patients with local inflammation (ACPA+/MRI+) did express higher erosion scores than ACPA-negative patients without local inflammation (ACPA–MRI–; $p<0.001$; Figure 1C). Furthermore, comparing ACPA-positive patients without local inflammation (ACPA+MRI–) to ACPA-positive patients with local inflammation (ACPA+MRI+) revealed that the latter group had significantly

higher erosions scores (p=0.016, Figure 1C). This suggests that ACPA with concomitant inflammation, but not ACPA 'alone', associated with higher erosion scores.

When 'any inflammation' (considering inflammation positive if: either local subclinical joint inflammation was present, or CRP was elevated) was studied, stratified analyses revealed similar results (Supplementary Figure 1). Also here, patients that had ACPA as well as inflammation present had higher erosion scores, in contrast to patients that had ACPA without concomitant inflammation (p=0.056). ACPA-levels within ACPA-positive patients (comparing tertiles) were not associated with erosion score (Supplementary Figure 2).

Mediation analyses; local inflammation is in the causal path of ACPA and erosions

In three steps, it was studied if local inflammation is intermediary in the causal path of ACPA and erosions using mediation analyses. In linear regression analysis (Figure 2), the presence of ACPA significantly associated with erosion score (β 0.72; 95%CI 0.23-1.2; p=0.004). Likewise, presence of ACPA also significantly associated with the severity of local inflammation (β 3.2; 95%CI 1.8-4.6; p<0.001). Importantly, presence of ACPA no longer showed a significant effect on erosion score when corrected for inflammation (β 0.31 ; 95%CI -0.15-0.77; p=0.18). Together, these results indicate that subclinical inflammation is a mediator acting in the causal path of ACPA leading to erosions and the mediator could account for more than half of the total effect: (A*B) / (A*B + C') = 0.57.

ACPA in the presence of RF, but not ACPA alone, associated with higher erosion scores

ACPA-positive patients with local inflammation were more often RF-positive (81%) than ACPA-positive patients without local inflammation (67%, p=0.28). Stratification was therefore applied for ACPA and RF:

studying the combinations of ACPA-positivity and RF-positivity revealed that ACPA-positive/RF-negative patients had similar erosion scores as ACPA-negative/RF-negative patients (p=0.30). However, patients having both ACPA and RF had significantly higher erosion scores (p=0.006; Figure 1D) as compared to ACPA-negative/RF-negative patients.

Finally, triple stratification for ACPA, RF and local subclinical joint inflammation was performed (Figure 3). First, we investigated if the single presence of ACPA or RF was associated with higher erosion scores. As compared to the ACPA–RF–MRI– reference group (median erosion score 1.0), no differences were found for patients only positive for ACPA (ACPA+RF–MRI–; median 1.0; p=0.85), nor single-positive for RF (ACPA–RF+MRI–; median 0.5; p=0.35) or patients positive for both ACPA and RF, but without subclinical joint inflammation (ACPA+RF+MRI–; median 1.0; p=0.65). ACPA+RF+MRI- patients (median 1.0) did not have significantly higher erosion scores than ACPA-RF+MRI- patients (median 0.5; p=0.91). We then investigated if erosion scores were significantly higher if concomitant inflammation was present in addition to the presence of ACPA and/or RF. Compared to ACPA–RF–MRI– patients, significantly higher erosion scores were observed for ACPA-positive patients with concurrent inflammation (ACPA+RF–MRI+; median 2.0; p=0.033), as well as RF-positive patients with concomitant inflammation (ACPA–RF+MRI+; median 2.25; p=0.001). Finally, we studied the erosion scores in ACPA+RF+ patients. Whereas ACPA+RF+MRI- patients did not have higher erosion scores than the reference group, ACPA+RF+MRI+ patients did have higher erosion scores than the ACPA–RF–MRI– patients (median 2.5 versus 1.0; p<0.0001). The erosion score of the ACPA+RF+MRI+ patients was also higher than that of the ACPA+RF+MRI– patients (median 2.5 versus 1.0; p=0.039). Together these data showed that the presence of ACPA and/or RF is only associated with higher erosion scores if concomitant inflammation is present.

Discussion

This study evaluated associations between ACPA, RF, (local) subclinical joint inflammation and erosions in arthralgia patients at risk for RA. Presence of ACPA alone, without inflammation, was not associated with higher erosion scores, in contrast to the combined presence of ACPA and inflammation. Mediation analyses revealed that local inflammation was intermediary in the causal path to erosions. These results indicate that joint inflammation has a role in the development of erosions in ACPA-positive individuals, and suggest that *in vitro* or mice model findings on the independent effect of ACPAs on erosions are in contrast to findings in humans.

Furthermore, the combination of ACPA and RF, rather than presence of ACPA alone, associated with erosions in arthralgia patients. These results align with those obtained in patients with early rheumatoid arthritis[8,9] and fuel the hypothesis that ACPAs alone are not the main and/or single pathogenic factor contributing to joint erosions. Although one can speculate how –or if– ACPAs contribute to joint erosions together with inflammation, results from association studies do not allow conclusions on biological mechanisms.

Our results suggest that, in addition to ACPA, local joint inflammation is required for more severe erosive disease. Based on the mediation analysis we cannot definitely differentiate between full or partial mediation; the significance for ACPA from step 1 was lost in step 3 suggesting full mediation. However, as the beta was not zero, partial mediation cannot be excluded. Nonetheless, results of the mediation analyses supported the notion that erosions in ACPA-positive arthralgia rarely occurred without concomitant inflammation. This finding is in line with a previous study that showed that increased levels of CD19+ B cells and CXCL13 were observed in ACPA-positive RA and were associated with erosive disease[18].

In this study, the use of sensitive high-quality MRI-data allowed us to detect erosions in a population in which the total burden of erosions is relatively low. In contrast to the setting of early inflammatory arthritis where all patients have current or recent joint inflammation, the arthralgia setting allows comparison of patients with and without inflammation.

Not all patients considered at risk for RA will develop arthritis over time, even though ACPA or (subclinical) inflammation might be present. However, because we addressed whether ACPA can directly mediate bone loss with/without concurrent inflammation, the study could be performed independent of the final clinical diagnosis.

The obtained subgroups after stratification were small in some cases (especially the ACPA+RF–MRI– subgroup after triple stratification), which could lead to underpowered analyses and the possibility of a lack to find statistically significant differences. However, all analyses show that erosion scores are highest when both ACPA and inflammation are present simultaneously which strengthens the overall findings.

Finally, our study cannot address the question if the results are different for specific ACPA reactivities, as the presence of ACPA was evaluated using the commercially available CCP2 test.

Because a direct effect of ACPA on erosions has been suggested[5], we studied erosions in humans. Although loss of trabecular bone as observed in mice may be dissimilar from periarticular-located erosions in humans, including the underlying mechanisms, our results indicate that ACPAs do not directly contribute to the formation of bone erosions, one of the hallmarks of RA.

Conclusions

In conclusion, the present data in patients with arthralgia showed that erosions are associated with the combined presence of ACPA and RF, rather than with ACPA alone, and preferentially occur in patients with joint inflammation.

Supporting information

Supplementary data is available at the website of Arthritis Research & Therapy, or can be obtained by contacting the first author.

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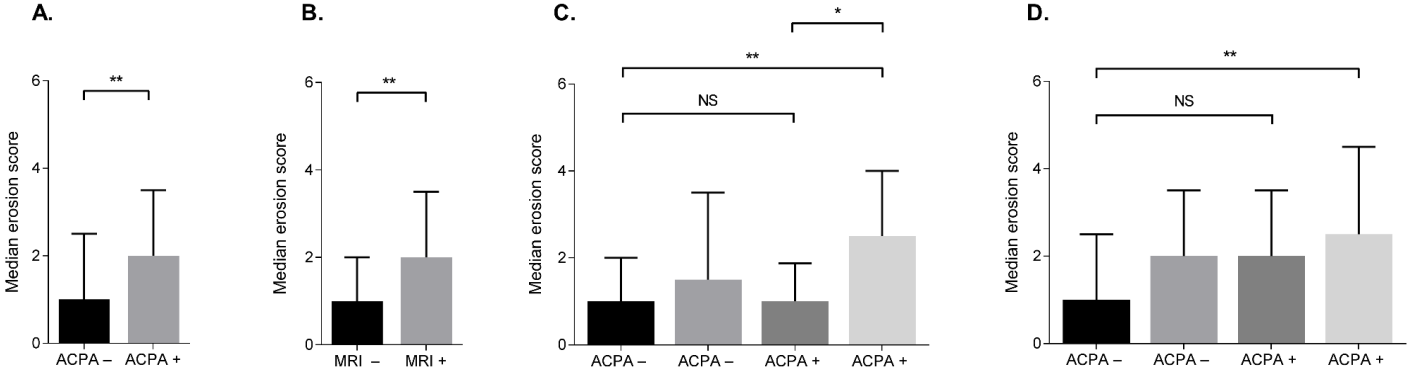
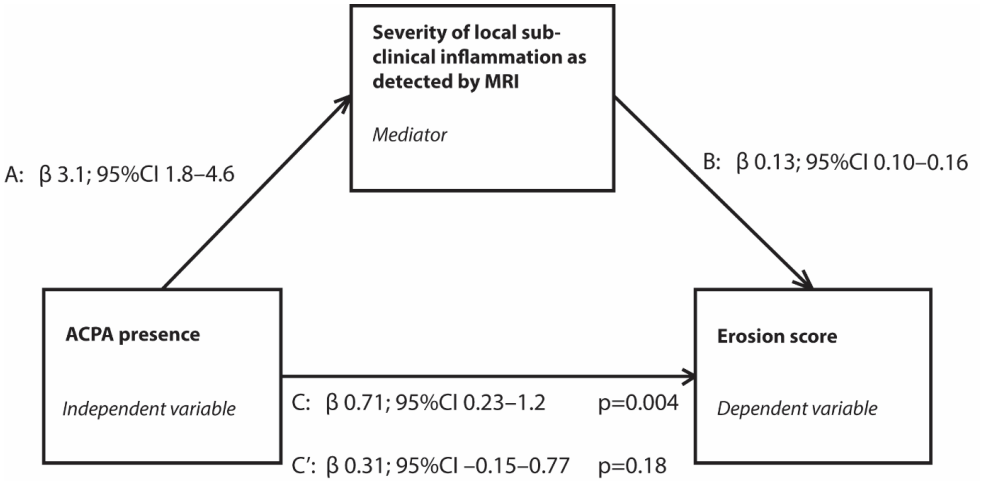
Figure 1. Histograms showing median erosion scores of patients with Clinically Suspect Arthralgia comparing ACPA-positive and ACPA-negative patients (A), patients positive or negative for local subclinical joint inflammation (B), ACPA-positivity and -negativity in relation to the concomitant presence of MRI-detected subclinical inflammation (C), or rheumatoid factor (D).



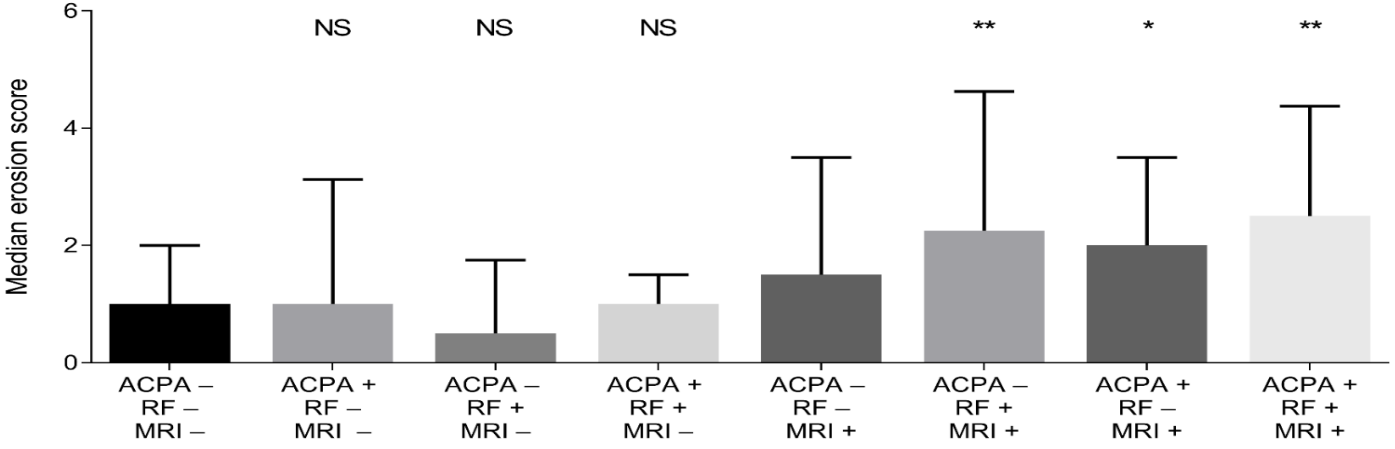
Legend:
Histograms showing median erosion scores with the upper limit of the interquartile range (75th percentile).** indicates significance of p<0.01 level, * indicates significance of p<0.05 level, NS indicates non-significance. The following comparisons have been made: ACPA+ vs. ACPA– (Figure 1A; p=0.006) and MRI+ vs. MRI– (Figure 1B; p<0.001). Next, ACPA+MRI– vs. ACPA–MRI– patients (Figure 1C; p=0.68), ACPA+MRI+ vs. ACPA–MRI– patients (Figure 1C; p<0.001) and finally ACPA+MRI– vs. ACPA+MRI+ (Figure 1C; p=0.016). ACPA+RF– patients vs. ACPA–RF– patients (Figure 1D; p=0.30) and ACPA+RF+ patients vs. ACPA–RF– patients (Figure 1D; p=0.006).

Figure 2. Mediation analyses showing that inflammation is in the causal pathways of ACPA.



Legend:
Schematic overview of the causal paths that were studied using mediation models as described by Baron and Kenny. The diagram illustrates the two causal paths that can lead to the outcome; a direct path from the independent to the outcome (C) and an indirect path from the mediator to the outcome (B). Finally, a path exists from the independent variable to the mediator (A). According to the description of Baron and Kenny, to test for mediation the following three regression analyses need to be performed[16]. (1) Regress the mediator on the independent variable (A); the independent variable should significantly affect the mediator. (2) Regress the dependent (outcome) variable on the independent variable (C); also here the independent variable should significantly affect the outcome. (3) Regress the dependent variable on both the mediator and the independent variable (B and C′ in one model); in case of mediation the mediator is significantly associated with the outcome and the effect of the independent variable on the outcome is less than in step 2 (partial mediation) or there is no effect at all (full mediation). In this study, the hypothesis was tested whether severity of local inflammation detected with MRI acts as a mediator in the causal path of the presence of anti-citrullinated protein antibodies (ACPA) on the erosion score. The data revealed that inflammation mediated the effect of ACPA on bone erosions. The mediator could account for more than half of the total effect: (A*B) / (A*B + C′) = 0.57.

Figure 3. Histograms showing median erosion scores of patients with Clinically Suspect Arthralgia with triple stratification for ACPA, RF and local joint inflammation.



Legend:

Histograms showing median erosion scores with the upper limit of the interquartile range (75th percentile). * indicates significance of p<0.01 level as compared to the ACPA–RF–MRI– group, ** indicates significance of p<0.01 level as compared to the ACPA–RF–MRI– group, NS indicates non-significance as compared to the ACPA–RF–MRI– group. The following comparisons were made: ACPA–RF–MRI– patients (median erosion score 1.0) vs. ACPA+RF–MRI– (median 1.0; p=0.85), ACPA–RF–MRI– vs. ACPA–RF+MRI– (median 0.5; p=0.35) and ACPA–RF–MRI– vs. ACPA+RF+MRI– (median 1.0; p=0.65). ACPA+RF+MRI- patients (median 1.0) vs ACPA-RF+MRI- patients (median 0.5; p=0.91). Next, ACPA–RF–MRI– patients were compared to ACPA+RF–MRI+ (median 2.0; p=0.033) and ACPA–RF+MRI+ patients (median 2.25; p=0.001). Finally, ACPA+RF+MRI+ patients were compared to ACPA–RF–MRI– patients (median 2.5 versus 1.0; p<0.0001), as well as ACPA+RF+MRI– patients (median 2.5 versus 1.0; p=0.039). The number of patients in each group was as follows: ACPA–RF–MRI– (n=214), ACPA+RF–MRI– (n=4), ACPA–RF+MRI– (n=26), ACPA–RF–MRI+ (n=174), ACPA+RF+MRI– (n=8), ACPA–RF+MRI+ (n=24), ACPA+RF–MRI+ (n=11) and ACPA+RF+MRI+ (n=46).

Table 1. Baseline characteristics of the Clinically Suspect Arthralgia patients (N=507).

Patient characteristic		
Age in years, mean (SD)	44	(13)
Female sex, N (%)	390	(77)
Family history of RA, N (%)	147	(29)
Symptom duration in weeks, median (IQR)	17	(9 – 32)
Presence of morning stiffness ≥60 minutes, N (%)	182	(36)
Current smoker, N (%)	137	(27)
68-TJC, median (IQR)	6	(3 – 10)
Increased CRP (≥5 mg/L), N (%)	106	(21)
Presence of local subclinical joint inflammation, N (%)	255	(50)
Positive for EULAR-definition for arthralgia suspicious for progression to RA [17], N (%)	325	(64)
Autoantibody status		
Negative for IgM-RF and ACPA, N (%)	385	(76)
IgM-RF-positive (≥3.5 IU/mL), ACPA-negative, N (%)	52	(10)
ACPA-positive (≥7 U/mL), IgM-RF-negative, N (%)	15	(3)
IgM-RF-positive and ACPA-positive, N (%)	55	(11)
ACPA-level (U/ml) in ACPA-positive patients, median (IQR)	162	(35 – 340)
ACPA-level (U/ml) in ACPA-positive patients without local joint inflammation, median (IQR)	129	(23 – 340)
ACPA-level (U/ml) in ACPA-positive patients with local joint inflammation, median (IQR)	191	(38 – 340)

Legend:
ACPA = anti-citrullinated peptide antibody; CRP = C-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; local subclinical joint inflammation = prevalence of MR-detected Bone Marrow Edema, synovitis or tenosynovitis was higher than that of age-matched symptom-free controls; TJC = tender joint count.

Chapter 7

Development of clinically apparent synovitis - a longitudinal study at joint level during progression to Inflammatory Arthritis

R.M. ten Brinck[1], H.W. van Steenbergen[1], A.H.M. van der Helm–van Mil[1,2]

1. Department of Rheumatology, Leiden University Medical Centre, Leiden, the Netherlands
2. Department of Rheumatology, Erasmus Medical Centre, Rotterdam, The Netherlands

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Abstract

Introduction: Subclinical inflammation, detected by Magnetic Resonance Imaging (MRI), in patients with arthralgia is predictive for development of inflammatory arthritis (IA). However, within patients that develop IA, the course of inflammation at joint level during this transition is unknown. This longitudinal study assessed progression of inflammation at joint level.

Methods: 350 joints (unilateral MCPs, wrist, MTP-joints) of 35 patients presenting with Clinically Suspect Arthralgia (CSA) that progressed to IA were studied at presentation with CSA and subsequently when clinical synovitis was first identified at joint examination (median time-interval 17 weeks). At both time-points, subclinical inflammation (bone marrow edema, synovitis, tenosynovitis) was evaluated with MRI and joint examination performed.

Results: At presentation with CSA, 71 joints showed subclinical inflammation. During progression to IA, 20% of these joints had resolution of inflammation, 60% had persistent inflammation and 20% progressed to clinical synovitis. Of all joints that had developed clinical synovitis (n=45), no prior subclinical inflammation was detected in 69%. Similar results were observed for ACPA-positive and ACPA-negative patients.

Conclusions: This longitudinal study demonstrated moderate correlations between joints with subclinical inflammation and joints that developed clinical synovitis. These data imply that IA development is a more systemic rather than a locally outgrowing process.

Introduction

During pre-arthritis phases of rheumatoid arthritis (RA), subclinical inflammation can already be present.[1-3] Its presence in small joints in patients with arthralgia is predictive of inflammatory arthritis (IA) development.[1-3] Furthermore, on patient-level, progression to IA is uncommon in arthralgia patients without MRI-detected subclinical inflammation.[1]

Although risk factor studies have made an enormous progress in our comprehension on the development of RA[4-6], many questions remain unanswered. One of these questions concerns the progression of inflammation on joint level during development of clinical synovitis. For instance, it is unknown how often joints with subclinical inflammation progress to clinical synovitis in the same joint, and vice versa, how often joints with clinical synovitis had (prolonged) preceding subclinical inflammation at the same location during the phase of arthralgia. Consequently, it is unclear whether IA development is a local outgrowing process where subclinical joint inflammation closely relates to subsequent clinical synovitis, or whether there is a more global deregulation where locations of subclinical inflammation and synovitis development are largely uncoupled. Exploration of these hypotheses necessitates longitudinal studies that start in a pre-arthritis phase.

This longitudinal study at joint level in arthralgia patients that developed IA assessed the course of joint inflammation in this period. In sensitivity analyses, stratification was applied for anti-citrullinated protein antibodies (ACPA) status.

Methods

Patients

350 small joints of 35 patients that presented with Clinically Suspect Arthralgia (CSA) and progressed to IA were evaluated. Patients presented at the rheumatology outpatient clinic of the Leiden University Medical Centre were included in a consecutive manner in the Leiden CSA cohort between April 2012 and September 2016. CSA was defined as recent-onset (<1 year) arthralgia of small joints that was clinically considered at risk for IA by the rheumatologists without clinically evident arthritis. 29 patients (83%) met the EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis at baseline.[7] Patients included in the CSA-cohort were followed until synovitis development (detected at joint examination by an experienced rheumatologist) as described in [8]. In this study, CSA-patients that were included between April 2012–September 2016 and progressed to IA were studied. Regular follow-up visits in the CSA-cohort were planned at 4, 12 and 24 months after baseline. 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. This provided early access to rheumatology care if patients developed clinically evident synovitis and thus inflammatory arthritis was identified at the first opportunity.

When IA was identified at patient level, individual joints could be in one of these states: clinical synovitis, MRI-detected subclinical inflammation but no clinical synovitis, or no inflammation. Tender joint count (68-TJC) and swollen joint count (66-SJC) for study purposes were performed by one assessor from a pool of six trained research nurses under supervision of an experienced rheumatologist. Regular reliability sessions are held to maintain a high interobserver correlation. All patients provided written informed consent. Ethics approval was provided by the local medical ethical committee under Ethics Approval Number NL38832.058.11.

MRI

Unilateral MRIs of wrist, MCP2–5, and MTP1–5 were performed at presentation with CSA (most painful side) and at first presentation with clinical synovitis (similar side as scanned at baseline). An ONI MSK Extreme 1.5T MRI scanner (GE Healthcare, Wisconsin, USA) was used, as described previously[1] and in the Supplementary Methods. Patients were instructed not to use NSAIDs 24 hours prior to MRI, with seven patients (20%) reporting daily use of NSAIDs at baseline. MRIs were evaluated for bone marrow edema (BME; range 0–72)) and synovitis (range 0–33) as described in [9], and tenosynovitis (range 0–54) as described in [10] by two independent experienced readers who were blind to clinical data and the order in time (all had interclass correlations ≥0.94, see Supplementary Table 1). Subclinical inflammation was considered present in clinically non-inflamed joints if the total inflammation score (summing the BME, synovitis and tenosynovitis scores and averaging the score of two readers) was ≥1. In other words: if either the BME, synovitis or tenosynovitis score was ≥1, subclinical inflammation was considered present in a joint. BME scores for the wrist joint were calculated for the bones lining the joint space: proximally the radius and ulna, distally the proximal carpal row (scaphoid, lunate, triquetrum and pisiform). Synovitis scores for the wrist joint were calculated by the radioulnar and radiocarpal compartment and tenosynovitis scores for all wrist flexors and extensors.

Analysis

Percentages were determined by evaluating inflammation in individual joints over time. Generalized Estimating Equations (GEE), using an unstructured correlation matrix, were used to investigate differences in time interval between the paired measurements in joints that did/did not develop clinical synovitis, while holding in account that one patient contributed 10 joints. Sensitivity analyses were performed per inflammatory feature and by stratifying for ACPA status. Finally, a second (more stringent) definition was used for subclinical inflammation:

subclinical inflammation was considered present if it occurred in <5% of age-matched symptom-free persons at the same joint and for the same feature (henceforth referred to as "5% corrected definition").

Results

Patient characteristics

Clinical characteristics are demonstrated in Table 1. Ten patients were ACPA-positive. 34 out of 35 patients (97%) had subclinical inflammation in ≥1 joint that was evaluated with MRI at baseline. Median duration between presentation with CSA and development of IA was 17 weeks (interquartile range (IQR): 6–21). When clinical synovitis was identified at one of the subsequent visits, the median swollen joint count (66-SJC) was 2 (IQR: 1–5), and 23 patients (66%) fulfilled the 2010 ACR/EULAR classification criteria for RA.

Joints with subclinical inflammation during CSA predominantly remain in state of subclinical inflammation

Further analyses were performed at joint level. At presentation with arthralgia, 71 out of 350 joints showed subclinical inflammation on MRI (Figure 1). Over time, 14 of these 71 joints (20%) had resolution of subclinical inflammation, 43 joints (60%) had persistent subclinical inflammation, and 14 joints (20%) progressed to clinical synovitis. 279 joints (80%) had no subclinical inflammation at baseline imaging.

Next, the absolute total inflammation scores were evaluated. The mean change in total inflammation score for all patients was 2.0 points (p=0.008). Summing BME, synovitis, tenosynovitis scores (yielding the total inflammation score) of the 43 joints that had subclinical inflammation at both time-points revealed that 16 joints (37%) had increasing inflammation scores (mean increase 1.8 points), 21 joints (49%) had identical

inflammation scores and 6 joints (14%) with subclinical inflammation had decreasing scores (mean decrease 1.4 points) despite still having scores >0 for subclinical inflammation.

Most joints developing clinical synovitis had no preceding subclinical inflammation at presentation with CSA

In total, 45 MCP, wrist or MTP-joints developed clinically apparent synovitis in 21 patients; 20 joints in the feet (MTP) were swollen, whereas 25 joints in the hand (MCP or wrist) were swollen. The other 14 patients had synovitis in ≥1 joint, but these joints were not evaluated on MRI. Of these 45 swollen joints, 31 joints (69%) had no preceding subclinical inflammation at presentation with arthralgia (Figure 1). A GEE investigating if swollen joints with or without preceding subclinical inflammation could have dissimilar times to arthritis revealed no difference in time intervals (β=1.3; p=0.71). Hence, the absence of preceding subclinical inflammation in joints with clinically apparent synovitis was not associated with a longer time interval. An MRI-example of a joint developing clinical synovitis whereas it showed no subclinical inflammation in the CSA-phase is presented in Figure 2A.

Analyses were repeated per inflammatory feature, revealing that 9% of joints (4 out of 45) with clinical synovitis had preceding BME in the CSA-phase. Similarly, 24% (11 out of 45 joints) had prior MRI-detected synovitis. For tenosynovitis, only the MCP and wrist joints were evaluated: 36% of joints (9 out of 25) had preceding tenosynovitis (Supplementary Figure 1A–C).

Resolution of subclinical inflammation was observed despite progression to IA

14 joints (in 11 different patients) had subclinical inflammation in the CSA-phase which resolved over time, despite progression to IA at patient-level: an MRI-example is provided in Figure 2B.

However, the majority of joints assessed (N=227/350; 65%) had no inflammation at either point in time (Figure 1).

Similar results observed for joints of ACPA-positive and ACPA-negative patients

As the pathogenesis of IA development presumably differs between ACPA-positive and ACPA-negative disease, analyses were stratified for ACPA status. On patient-level, the interval between presentation with CSA and IA development was shorter for ACPA-positive disease (median 7 weeks, compared to 18 weeks in ACPA-negative disease). However, at joint level the percentages of joints that progressed from subclinical inflammation to clinical synovitis were similar (Supplementary Figure 2B). Likewise, analyses within ACPA-positive disease showed that 62% of joints with clinically apparent synovitis had no prior subclinical inflammation in the same joint, whereas in ACPA-negative disease this percentage was 72%.

Inflammation corrected for age-matched symptom-free persons yielded similar results (5% corrected definition)

Finally, a second definition of presence of MRI-detected subclinical inflammation was used. The values of normality of MRI-detected joint inflammation depends on age and should take into account the occurrence of inflammation in symptom-free persons. Therefore, in this second definition (5% corrected definition), subclinical inflammation was considered present after correction for the level of inflammation occurring in <5% of age-matched symptom-free persons at the same joint and for the same feature (a definition used previously [1,13]). With the 5% corrected definition, similar findings were obtained (Supplementary Figure 3), with the majority of small joints (84%) that developed clinical synovitis having no preceding phase lasting for weeks with subclinical inflammation in the same joint.

Discussion

To our knowledge, this study was the first to perform longitudinal joint-level analyses in order to investigate progression of inflammation in patients converting from CSA to the earliest clinical phase of IA. On joint level, only moderate correlations were observed between presence of subclinical inflammation and subsequent development of clinical synovitis. The majority of joints with clinical synovitis had no subclinical inflammation in the same joint at the baseline observation.

The present joint-level observations on inflammatory progression fit best with the hypothesis of 'global deregulation', rather than that of a localized exacerbating process. Previous observations of increased markers of systemic inflammation in pre-arthritis phases may support this.[11] Additionally, our study showed only moderate correlations between inflammation on MRI and progression of synovitis as assessed by physical examination, with BME showing the lowest proportion of prior subclinical inflammation in the joints progressing to synovitis. Another study found that MRI-detected subclinical inflammation on is not only present in clinically swollen but also in non-swollen joints; in particular, BME frequently occurred in clinically non-inflamed joints.[12]

Our results should be interpreted within the context of some methodological limitations. Considering that 10 joints (unilateral MCP, wrist and MTP-joints) per patient were studied, many synovial joints were not assessed. Furthermore, the total sample size was limited despite large availability of data on joint level. However, this study offers the first and largest longitudinal scrutiny of data on joint inflammation in arthralgia patients that progress to IA. Finally, the time interval between presentation with CSA and IA differed between individual patients, posing the possibility of dissimilarities in time intervals for joints with or without preceding subclinical inflammation. Nonetheless, a GEE model

incorporating each patient contributing 10 joints suggested no differences in time to arthritis for joints with or without preceding subclinical inflammation, indicating that results were not based on a few patients with longer time intervals.

An elementary, but sensitive, definition of subclinical inflammation (summed inflammation-score ≥1 per joint) was used. Reference values of normality for MRI-detected joint inflammation depending on age, inflammatory feature and joint were not included in this definition. When subclinical inflammation was defined as inflammation present in <5% of age-matched symptom-free persons at the same joint and for the same feature (5% corrected definition), similar findings were obtained.

ACPA-positive and ACPA-negative disease have different risk factors, presumed differences in pathogenesis, and known dissimilarities in speed in progressing from CSA to IA.[14] However despite these differences, the observations on joint level on the relation between subclinical inflammation in the CSA-phase and clinical synovitis in the IA-phase were roughly similar in both groups. Larger studies validating these findings are required.

MR-imaging depicts inflammation of different tissues around the joint. Stratified analyses showed that clinical synovitis was most often preceded by MRI-detected tenosynovitis. This fits previous observations that tenosynovitis had the highest predictive accuracy for RA development[1], and that tenosynovitis was an initial preclinical change in mouse models of arthritis development.[15] Although further studies are needed to explore this thoroughly, the combination of these findings suggests that tenosynovitis is a very early phenomenon.

The ability of a clinician to detect swollen joints may be dissimilar between different joint groups (MCP, wrist and MTP joints) analysed in this paper.

Nevertheless, the number of swollen joints in the feet (MTP: 20 joints) was similar to the number of swollen joints in the hand (MCP and wrist: 25 joints). Future studies could investigate the correlation between MRI-detected subclinical inflammation in clinically swollen and non-swollen joints in the feet versus joints of the hand.

This study evaluated inflammation on MRI-scans in individual joints over time. Future studies with serial MR-imaging at more frequent time-points (and thus shorter intervals) in patients progressing from arthralgia to RA might further increase the understanding of inflammatory processes in small joints during IA development.

In conclusion, this first longitudinal MRI study on joint level during progression from CSA to IA indicates that the course of subclinical inflammation is variable and showed that the majority of small joints that developed clinical synovitis had no subclinical inflammation in the same joint at the baseline observation.

Supporting information

Supplementary data is available at the website of RMD Open, or can be obtained by contacting the first author.

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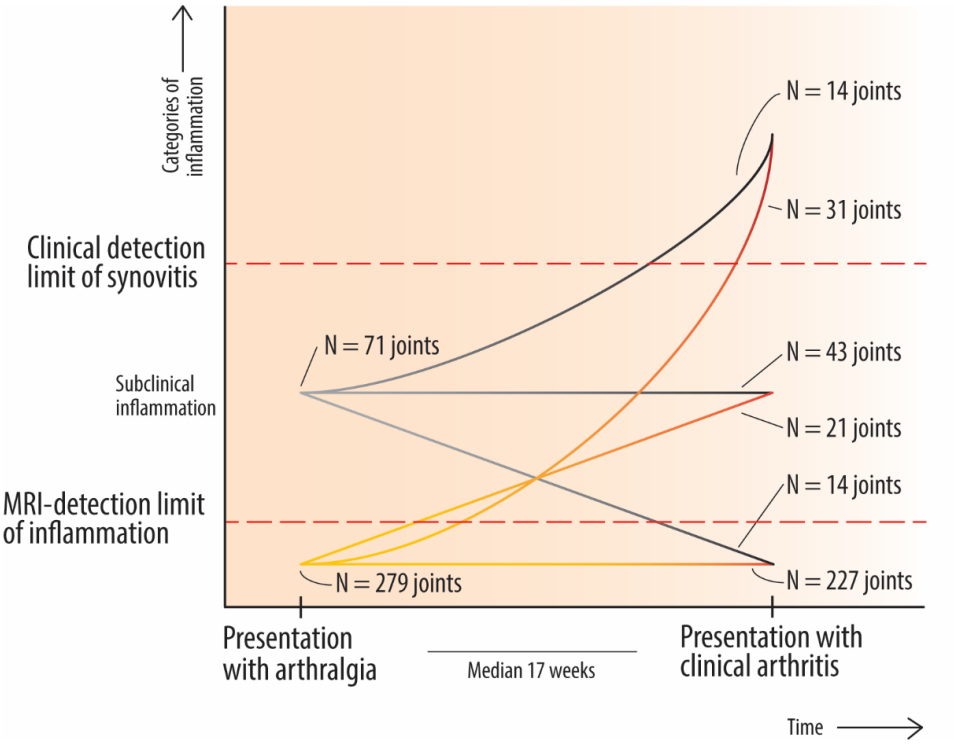
Table 1. Characteristics of the 35 patients at the time of presentation with arthralgia and at the consequent time of IA development

Characteristic		
Presentation with arthralgia		
Age in years, mean (SD)	44	(13)
Female sex, N (%)	25	(71)
Family history of RA, N (%)	12	(39)
Tender joint count (68-TJC), median (IQR)	5	(4 – 10)
Increased CRP (≥5 mg/L), N (%)	6	(19)
Symptom duration (weeks), median (IQR)	17	(9 – 31)
Autoantibody status		
Negative for IgM-RF and ACPA, N (%)	19	(54)
Single IgM-RF-positive (≥3.5 IU/mL), N (%)	6	(17)
Single ACPA-positive (≥7 U/mL), N (%)	2	(6)
Positive for IgM-RF and ACPA, N (%)	8	(23)
At development of inflammatory arthritis		
Fulfilled 2010 ACR/EULAR RA classification criteria, N (%)	23	(66)
Swollen joint count (66-SJC), median (IQR)	2	(1 – 5)
1 swollen joint, N (%)	10	(29)
2 swollen joints, N (%)	8	(23)
≥3 swollen joints, N (%)	17	(49)

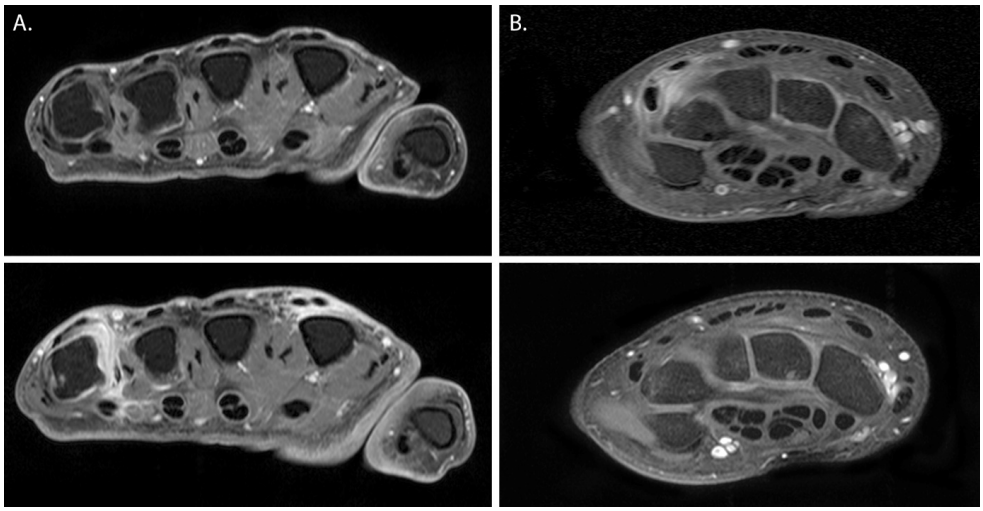
Legend:
ACPA = anti-citrullinated peptide antibody; CRP = C-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; SJC = swollen joint count; TJC = tender joint count.

Figure 1: Schematic depiction of categories of inflammation in 350 small joints during progression from Clinically Suspect Arthralgia to Inflammatory Arthritis



Legend:
Presence of subclinical joint inflammation was detected by Magnetic Resonance Imaging. An MR inflammation score ≥1 (sum of BME, synovitis or tenosynovitis in one joint, average of two readers) was defined as subclinical inflammation. At the time of IA development, individual joints could show clinically detectable joint swelling at physical examination, while this was per definition not possible at presentation with arthralgia. Please note that the Y-axis indicates categories of inflammation (below MRI-detection limit of inflammation / above MRI-detection limit but under clinical detection limit of synovitis at physical examination / above clinical detection limit of synovitis at physical examination (i.e. clinically detectable joint swelling)), not absolute MRI-inflammation scores.

Figure 2: Examples of MR-imaging at presentation with CSA (top panel) and at IA development (bottom panel), showing joints that (A) from no inflammation to clinical synovitis and, (B) resolution of subclinical inflammation.



Legend:
Presented in A. are: (top panel) left MCP joints with no subclinical inflammation as detected by MRI, and (bottom panel) left MCP joints of the same patient with synovitis in MCP5 and tenosynovitis in MCP2 and 5. According to clinical examination the patient developed clinical synovitis in the left MCP2 (depicted), left MCP5 (depicted), left PIP2 and right PIP5 joints (both not imaged). From a different patient (B.) are presented: (top panel) right wrist joint with tenosynovitis in the extensor carpi ulnaris tendon, and (bottom panel) right wrist joint of the same patient without MRI-detected subclinical inflammation despite progression to inflammatory arthritis on patient-level. The patient developed clinically apparent synovitis in the left PIP3 joint (not imaged). All images were made in T1-weighted FSE sequence with frequency selective fat saturation in the axial plane after gadolinium contrast injection.

Chapter 8

Sequence of joint tissue inflammation during Rheumatoid Arthritis development

R.M. ten Brinck [1], H.W. van Steenbergen [1], A.H.M. van der Helm–van Mil [1,2]

1. Department of Rheumatology, Leiden University Medical Centre, Leiden, the Netherlands
2. Department of Rheumatology, Erasmus Medical Centre, Rotterdam, The Netherlands

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Abstract

Objective: Subclinical joint inflammation in patients with arthralgia is predictive for progression to rheumatoid arthritis (RA). However, the time course in which bone marrow edema (osteitis), synovitis, and/or tenosynovitis progress is unsettled. This longitudinal study assessed the course of MRI-detected subclinical joint inflammation during progression to RA.

Methods: Patients that progressed from Clinically Suspect Arthralgia (CSA) to RA underwent 1.5T MRI of MCP, wrist and MTP-joints at presentation with arthralgia and at first identification of synovitis assessed through physical examination (n=31). MRIs were evaluated for osteitis, synovitis, tenosynovitis, and erosions by two readers, blinded for clinical data and order in time. To estimate changes in MRI scores between the asymptomatic state and CSA-onset, scores of MRI-features at CSA-baseline were compared to scores from age-matched symptom-free persons.

Results: At presentation with CSA, synovitis and tenosynovitis scores were higher than scores from age-matched symptom-free persons (p=0.004 and p=0.001, respectively). ACPA-positive arthralgia patients also had increased osteitis scores (p=0.04). Median duration between presentation with arthralgia and RA development was 17 weeks. During progression to RA, synovitis and osteitis increased significantly (p=0.001 and p=0.036 respectively), in contrast to tenosynovitis and erosion scores. This pattern was similar in both ACPA-subsets, although statistical significance was reached for synovitis and osteitis in ACPA-negative, but not in ACPA-positive RA.

Conclusion: Increased tenosynovitis and synovitis scores at CSA-onset and the increase in synovitis and osteitis during progression to RA suggest an 'outside-in' temporal relationship of arthritis development; in particular for ACPA-negative RA. For ACPA-positive RA further studies are needed.

Background

Rheumatoid Arthritis (RA) can be diagnosed at the time patients present with clinically detectable inflammatory arthritis (swollen joints). It is known that immunological alterations are present long before that.[1] For instance, Magnetic Resonance Imaging (MRI)-detected subclinical inflammation is present weeks to months before arthritis becomes clinically apparent in patients presenting with Clinically Suspect Arthralgia (CSA) and has been shown predictive for RA development.[2] Nonetheless, a long-standing question is where inflammation starts in the joint, or in what order the different tissues of the joints (synovium, tenosynovium and bone) become inflamed during the development of RA.

Several hypotheses about the chronology of arthritis development prevail. Firstly, it has been postulated that synovitis is an initial process that is succeeded by bone involvement. This is the traditional 'outside-in hypothesis', presuming that inflammation of the synovium precedes bone marrow edema (or osteitis).[3-7] Alternatively, it has been suggested that RA is a primary bone marrow disease, which subsequently encroaches upon the synovial membrane; this is the 'inside-out hypothesis', with osteitis preceding synovitis, a hypothesis that has become popular after imaging and histological studies had revealed the presence of osteitis at locations with MRI-detected osteitis in patients with RA.[4,7,8] It has also been hypothesized that these processes could emerge and progress simultaneously with microscopic bone canals allowing transduction of inflammation from the outside (synovium) to the inside (bone marrow) and vice versa.[4,7,9] Finally, mouse models of arthritis development have shown that tenosynovitis was the initial preclinical change.[10] Extrapolation to humans would suggest that neither synovitis nor osteitis, but tenosynovitis is the primary feature of joint inflammation.[10] For the development of bone erosions (instead of the development of clinically apparent inflammatory arthritis) similar hypotheses have been postulated, with the primary inflammation located inside[11,12], or outside the bone

respectively.[13] Altogether, temporal relationships are yet unknown and can be discovered by longitudinal imaging studies that start in pre-arthritis phases of the disease.

To address the question on the chronological order in which the different tissues of the joints become inflamed, this longitudinal MRI study investigated the course of MRI-detected subclinical joint inflammation (synovitis, tenosynovitis, osteitis) and erosions in patients that presented with arthralgia and progressed to RA. MRIs were performed longitudinally at presentation with arthralgia and at development of RA. Although no MRIs were made in the asymptomatic phase of these patients, the MRI-data obtained at presentation with arthralgia were compared to data obtained in age-matched symptom-free persons, to estimate the course of inflammation before presentation with arthralgia. Finally, as anti-citrullinated protein antibodies (ACPA)-positive and ACPA-negative RA are considered as different disease subsets with differences in the underlying pathophysiology, analyses were stratified for ACPA-positive and ACPA-negative RA to explore if there are differences in the chronological order in which different articular tissues become inflamed.

Patients and Methods

Patients

We longitudinally followed patients that presented with Clinically Suspect Arthralgia (CSA) between April 2012-September 2016. The Leiden CSA-cohort consecutively included patients that presented at the rheumatology outpatient clinic of the Leiden University Medical Centre without clinically evident arthritis, but with recent-onset (<1 year) arthralgia of small joints that was considered at risk for RA by their rheumatologists based the clinical presentation.[14] A detailed description of the in- and exclusion criteria of the Leiden CSA-cohort and the study protocol are described in [15]. Absence of clinical arthritis at baseline was ascertained through physical examination by an experienced rheumatologist.

Regular follow-up visits in the cohort were planned at 4, 12 and 24 months. If necessary (for instance when the patient experienced more symptoms or noticed a swollen joint) patients were seen in between scheduled visits by their rheumatologist. Hence, logistics were arranged such that patients in this cohort had very easy access to rheumatology care and clinically evident inflammatory arthritis was identified at the first opportunity. The outcome was clinically apparent arthritis, identified at joint examination by an experienced rheumatologist. Previous studies revealed that ~20% of patients that presented with CSA progressed to clinically apparent inflammatory arthritis.[2]

For this study we selected the patients included in the CSA cohort that developed clinically apparent RA. A flowchart is provided in Figure 1. From a total of 416 patients included in the CSA-cohort during the studied period, 76 were diagnosed with RA. Serial MR imaging was performed at baseline and at arthritis development (but before DMARDs were started). Of the 76 RA-patients, serial MRIs were available in 35 patients. In 41 patients serial imaging was not available because: 4 patients had contra-indications for gadolinium contrast, 3 patients progressed to RA in a very short period of time and 34 patients were lacking serial MR imaging due to logistical reasons.

35 patients were diagnosed with RA and had serial MR imaging available. Four out of these 35 patients did not receive a recipe for a DMARD at the visit clinical arthritis was identified (3 patients subsequently had resolution of arthritis, 1 patients received a recipe the next visit). Thus, in total 31 patients with a clinical diagnosis of RA and immediate prescription of DMARDs were studied. Notably, fulfilment of the 2010 classification criteria was not required to define RA as ACPA-negative patients need >10 involved joints to fulfil these criteria[16], and this can be hampered by DMARD-initiation. Nonetheless, despite this theoretical threshold, 21 patients (68%) fulfilled the 2010 criteria for RA at arthritis development. It is important to note that DMARDs (including corticosteroids) were not

prescribed in the phase of arthralgia.

Symptom-free persons

Serial MRIs were not made in the period preceding presentation with CSA. To infer on the course of MRI-detected subclinical inflammation over time before the phase of CSA, the MRI data from the 31 CSA-patients were compared to data from symptom-free persons.[17] Symptom-free persons were matched based on age in a 1:2 ratio. These 62 symptom-free persons had no history of inflammatory rheumatic diseases, no joint symptoms during the last month and no evidence of synovitis at physical examination. The persons were retrieved from the general population; the recruitment procedure is described in [17]. Because gender (p=0.10), increased BMI (p=0.59) and smoking (p=0.21) had no effect on MRI-detected inflammation, matching was not performed on these factors.

MRI

Unilateral MRIs of wrist, MCP2–5, and MTP1–5 joints were performed at presentation with CSA (most painful side) and at first presentation with clinical synovitis (similar side as scanned at baseline). A total of 18 tenosynovitis locations were scored in each patient: 10 at the wrist, including 6 extensor compartments and 4 regions on the volar side (the flexor digitorum profundus and flexor digitorum superficialis, the flexor pollicis longus, the flexor carpi ulnaris, and the flexor carpi radialis), and 8 locations at MCP joints 2–5 (paired flexor tendons and extensor tendons of the fingers). An ONI MSK Extreme 1.5T MRI scanner (GE Healthcare, Wisconsin, USA) was used, as described previously[2] and in the Supplementary Methods. All patients were instructed not to use NSAIDs 24 hours prior to MRI, with only seven patients (23%) reporting the daily use of NSAIDs at baseline. The serial MRIs were scored blinded for clinical data and order in time by two readers for osteitis, erosions, and synovitis as described in [19], and tenosynovitis as described in [20]. Mean scores of two readers were studied. Additional information on the scoring

is provided in the Supplementary Methods. Three readers contributed to the scoring of the MRIs. Within-reader intraclass correlation coefficients (ICC) and between-reader ICC were all >0.90 and are also presented in Supplementary Table 1. Results of MR imaging were not shared with the treating rheumatologists.

Analyses

Paired t-tests were used. Longitudinal modelling was performed using Generalized Estimating Equations (GEE): the scores of the different inflammatory features and erosions were studied over time. The GEE models, utilizing an unstructured correlation matrix, corrected for the influence of age, gender and time to progression to RA and baseline score per feature. Residuals of GEE modelling were checked for normal distribution. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS, version 23.0). P-values <0.05 were considered significant.

Results

Patients

Characteristics of the 31 RA-patients studied were similar to the total group of 76 patients diagnosed with RA, except for a lower frequency of ACPA-positivity in the studied group (Supplementary Table 2). Baseline characteristics of the 31 patients studied are presented in Table 1. The mean age was 44 years, 71% was female, the median tender joint count (68-TJC) was 5 (interquartile range (IQR): 4–10), 42% was Rheumatoid Factor-positive and 29% was ACPA-positive. These characteristics are in line with previous research in CSA patients.[15] The median interval between presentation with arthralgia and progression to RA was 17 weeks. The characteristics of the 31 patients at the time of identification of RA are also presented in Table 1. The DMARDs that were initiated after RA development are presented in Supplementary Table 3.

MRI-data at presentation with arthralgia compared to that of age-matched symptom-free controls

Compared to age-matched symptom-free persons from the general population, CSA patients had increased tenosynovitis (mean 1.7 versus 0.27; $p<0.001$) and synovitis scores (2.2 versus 0.93; $p<0.001$; Figure 2). Osteitis scores (1.9 versus 1.4; $p=0.35$) and erosion scores (1.7 versus 1.8; $p=0.53$) were not significantly elevated (Figure 2) at presentation with arthralgia.

Inflammation increased during progression from arthralgia to rheumatoid arthritis

During progression from arthralgia to RA, the mean osteitis score increased from 1.9 to 2.7 ($p=0.036$), and the mean synovitis score from 2.2 to 3.4 ($p=0.001$; Figure 2). Tenosynovitis and erosion scores did not increase significantly (mean 1.7 to 2.0; $p=0.35$ and 1.7 to 1.9; $p=0.092$, respectively; Figure 2). Next, GEE models were constructed, allowing longitudinal data comparisons of MRI inflammatory scores. These models corrected for gender, age at inclusion, time interval to RA and the score of each feature (e.g. osteitis) at presentation with CSA. GEE modelling demonstrated that only synovitis scores ($\beta=1.0$; $p=0.004$) were significantly higher at time of RA development than at presentation with arthralgia.

Stratification for ACPA-status

Finally, it was explored if results were different in ACPA-positive and ACPA-negative RA patients, although absolute numbers were small (n=9 and n=22, respectively). ACPA-positive patients had higher osteitis scores ($p=0.04$), synovitis scores ($p<0.001$) and tenosynovitis scores ($p<0.001$) at presentation with arthralgia, as compared to age-matched symptom-free persons. ACPA-negative patients had higher synovitis scores ($p=0.02$) and tenosynovitis scores ($p=0.001$), but the osteitis score was not different ($p=0.24$). Erosion scores were not different from age-matched symptom-free persons in both ACPA-positive and ACPA-negative patients at presentation with arthralgia.

Over time, during progression from arthralgia to RA, ACPA-status did not change for any of the patients. In ACPA-negative patients, osteitis and synovitis score increased significantly (1.1 to 1.7; p=0.03 and 1.8 to 2.9; p=0.003, respectively) during progression from arthralgia to RA, whereas tenosynovitis and erosion scores remained stable (Figure 3). In ACPA-positive patients no statistical significance was obtained but osteitis scores increased from 3.6 to 5.2 (p=0.22), and synovitis from 3.1 to 4.5 (p=0.13). Tenosynovitis scores were 1.7 and 2.3 (p=0.32) and erosion scores were 2.4 and 2.6 at presentation with arthralgia and RA respectively (p=0.62; Figure 3).

Finally, analyses were repeated for autoantibody positive (positive for either RF and/or ACPA; n=16) and auto-antibody negative patients (n=15). Similar results were obtained as for ACPA-positive and ACPA-negative RA-patients as described above (data not shown).

Discussion

In order to increase the understanding of the chronological order of joint inflammation during the development of RA, serial MRIs were made in RA-patients at the time of presentation with arthralgia and at first identification of RA. This revealed that synovitis and osteitis increased during the symptomatic pre-arthritis phase. In addition, data on MRI-detected subclinical inflammation obtained at presentation with arthralgia were compared with data from age-matched symptom-free persons. This showed that synovitis and tenosynovitis scores were higher in patients with arthralgia, suggesting that these features had already increased at presentation with arthralgia. The erosion scores did not increase, neither in the asymptomatic nor during the symptomatic phase preceding the identification of RA. Together, these results suggest that tenosynovitis and synovitis are the earliest inflammatory features. Subsequently, synovitis and osteitis increased over time in the symptomatic pre-arthritis phase, whereas erosions did not yet increase before RA had become apparent.

This implies that inflammation mainly starts outside the bone (fitting the outside-in hypothesis), after which osteitis develops, which poses the joint at risk for structural damage development in the phase of clinically evident RA (if left insufficiently treated).

Stratification for ACPA-status showed that synovitis and osteitis progressed similarly during the symptomatic pre-arthritis phase in both ACPA-subsets, but that ACPA-positive arthralgia patients already had higher osteitis scores at presentation with arthralgia. This could be explained by a longer symptom duration of ACPA-positive patients at presentation with arthralgia: median symptom duration at baseline was 22 weeks in ACPA-positive CSA patients, and 15 weeks in ACPA-negative CSA patients (Supplementary Table 3). As established previously, ACPA-positive RA has a more gradual onset of symptoms.[21] ACPA-positive patients may, therefore, have presented in a slightly later phase of the disease. Alternatively, this finding could also imply a different chronology of joint inflammation in ACPA-negative and ACPA-positive RA, with osteitis being one of the first inflammatory features in ACPA-positive RA. This could be in line with the associations of autoantibody status and osteitis scores as observed in the phase of classifiable RA.[22]

There are several limitations to this study. A limitation is that the analyses were done on the sum of synovitis, osteitis and tenosynovitis scores observed in 310 joints, 1023 bones and 868 tendons. Whilst it would be interesting to perform similar analyses on individual joint/bone/tendon level, this was not done because of low numbers of joints/bones/tendons with subclinical inflammation. Larger studies are needed to this end.

Another limitation is the sample size. Although all patients that developed RA from a total cohort of consecutive CSA patients (with serial MR imaging available) were studied, the absolute number (n=31) is rather small. Future longitudinal imaging studies are therefore required to validate the results.

Nonetheless, the present study is the largest longitudinal MRI study in a population of patients that converted from arthralgia to RA that is available to date.

Analyses were stratified for ACPA to explore differences between these subgroup, although especially the ACPA-positive subgroup was rather small. This may be explained by more intramuscular corticosteroid injections in ACPA-positive patients, preventing the performance of an MRI before DMARD initiation. Larger future studies, especially in ACPA-positive convertors are needed.

Another limitations is that MRIs were made at first presentation with CSA and at development of RA, but no scans were made in between. Furthermore, patients were included at first presentation with clinically suspect arthralgia and were not scanned in an asymptomatic phase. Although some information on the chronology of joint inflammation in this disease phase was obtained by comparison of data obtained in age-matched symptom-free persons, this analysis was cross-sectional in nature and therefore less reliable than longitudinally collected data. Hence, future longitudinal imaging studies would be still highly relevant to further increase our understanding of RA development.

In our study, T1-weigthed fat suppressed sequences were obtained. These were previously demonstrated has a strong correlation with T2-weighted fat suppressed sequences in three studies.[23–25] Furthermore, persistent osteitis was strongly associated with erosive progression using these sequences.[11] Taken together, these findings demonstrate the osteitis is an established risk factor for the development of articular bone erosions regardless of the acquired sequence. The lack of finding a significant increase in osteitis scores between asymptomatic persons and arthralgia patients is more likely to be caused by the (early) disease stage in which the patients were assessed than a difference due to the scanning protocol.

Nevertheless, replication of our results using T2-weighted fat suppressed sequences is warranted.

Future serial imaging studies performed in the asymptomatic phase until development of RA would be useful to further elucidate the chronology in which the different articular tissues become inflamed. However, this cannot be easily accomplished as the 5-year positive predictive value of ACPA in the general population is approximately 5%.[26,27] Consequently, 20 symptom-free ACPA-positive persons would need to be followed for several years with serial MRIs to acquire longitudinal data of one RA patient. Studying 30 ACPA-positive RA patients already in the asymptomatic phase would require serial imaging in ~600 ACPA-positive symptom-free persons during several years of follow-up. Hence, although challenging, studying temporal relationships of inflammatory features during progression from the asymptomatic to the symptomatic pre-arthritis phase is a subject for follow-up research.

Animal studies have demonstrated that tenosynovitis was the earliest inflammatory joint feature in murine models.[10] Studies in CSA patients have also revealed that, of all three inflammatory features, tenosynovitis is the strongest predictor for RA development.[2] The current data, showing that tenosynovitis is a very early phenomenon, denotes the importance of inflammation of synovial tendon sheaths in very early phases of RA development.

The erosion score did not increase in the pre-arthritis phase, in contrast to the osteitis score. Previous research has revealed that osteitis that persisted in the early clinical phase of RA was strongly associated with erosion development (OR ~60) at the same location.[11] CSA-patients in this study had very early access to rheumatologic care in case of symptom deterioration or if they suspected joint swelling. Therefore, we expect that patients were seen very shortly after clinical synovitis had developed. In

addition, the median duration to development of RA was relatively short (17 weeks). Both factors may explain why articular bone erosions were not (yet) increased during the studied period. Based on MRI-data collected in early arthritis patients[11], we anticipate that the bones with osteitis at RA presentation were at risk for development of erosions, but the time period was too brief for actual erosive progression. This is in line with previous studies showing that MRI detected erosions predominantly developed in joints with persistent osteitis.[11]

Imaging using ultrasound (US) can be used to assess the presence of synovitis and tenosynovitis. Our data, obtained in the earliest disease phases, could suggest that US could suffice for assessment of these features. However, ultrasound cannot depict osteitis which may also provide valuable information. Studies in larger patient groups are needed before the present findings can be translated to clinical practice, though the present data contributes to our understanding of clinical arthritis and RA development.

Conclusions

In sum, the data provided on RA-patients suggests an increase of tenosynovitis and synovitis scores before the onset of arthralgia. Our study demonstrated that synovitis and osteitis scores increased during progression from arthralgia to clinical arthritis, suggesting an 'outside-in' temporal relationship of arthritis development, particularly in ACPA-negative RA. For ACPA-positive RA further studies are needed.

Supplementary information

Supplementary data is available at the website of Arthritis Research & Therapy, or can be obtained by contacting the first author.

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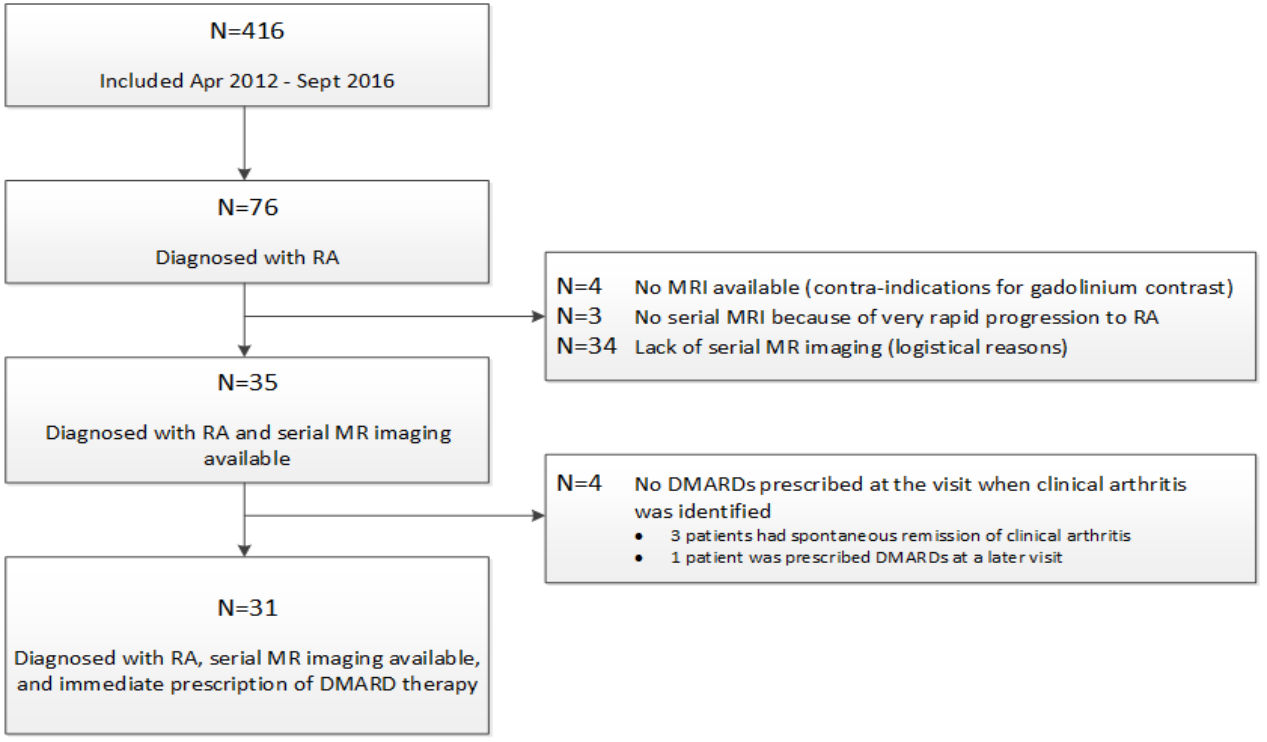
Table 1. Characteristics of the RA-patients at presentation with Clinically Suspect Arthralgia and at first identification of clinical arthritis and age-matched symptom-free volunteers

	At presentation with arthralgia (n=31)	At presentation with RA (n=31)	Age-matched symptom-free volunteers (n=62)
Age in years, mean (SD)	45 (14)	-- --	44 (13)
Female sex, n (%)	22 (71)	-- --	38 (61)
Symptom duration in weeks, median (IQR)	17 (9-32)	-- --	**n/a**
Presence of morning stiffness ≥60 minutes, n (%)	11 (35)	24 (77)	0 (0)
68-TJC, median (IQR)	5 (4–9)	7 (5–12)	0 (0–0)
66-SJC, median (IQR)	0 (0–0)	2 (1–5)	0 (0–0)
Increased CRP (≥5 mg/L), n (%)	6 (19)	7 (29)	**n/a**
Autoantibody status			
IgM-RF-positive (≥3.5 IU/mL), n (%)	13 (42)	13 (42)	**n/a**
ACPA-positive (≥7 U/mL), n (%)	9 (29)	9 (29)	**n/a**
Any antibody-positive (either RF or ACPA)	16 (52)	16 (52)	**n/a**

Legend:
ACPA = anti-citrullinated peptide antibody; CRP = C-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor; IQR = interquartile range; N/A = not applicable or not assessed; RA = rheumatoid arthritis; SD = standard deviation; SJC = swollen joint count; TJC = tender joint count.

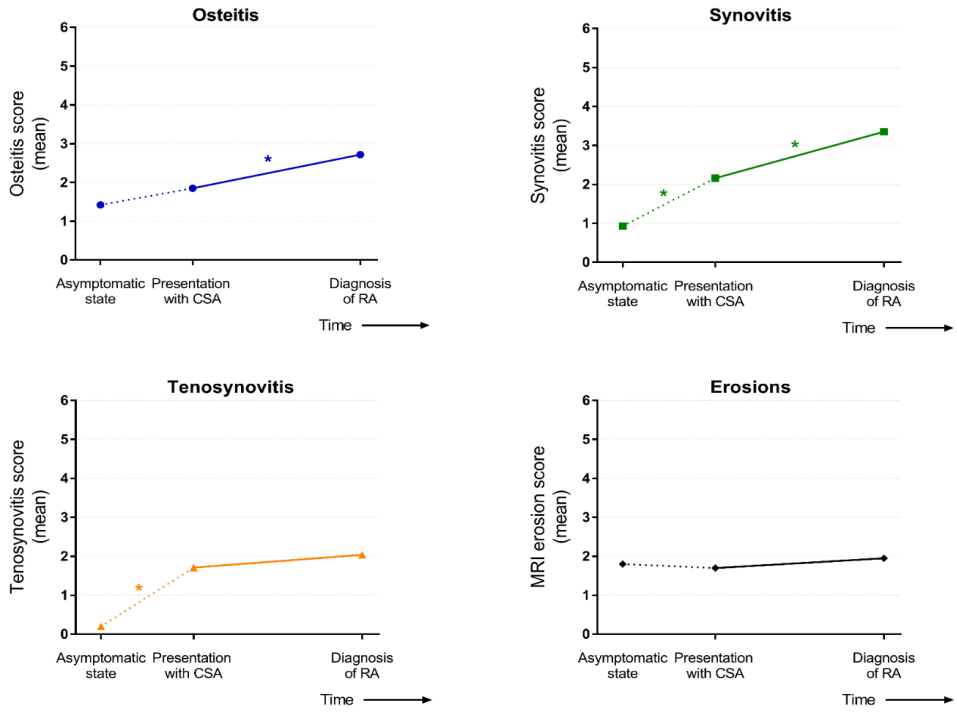
Figure 1. Flowchart of selection of RA patients studied from the total CSA cohort



Legend:

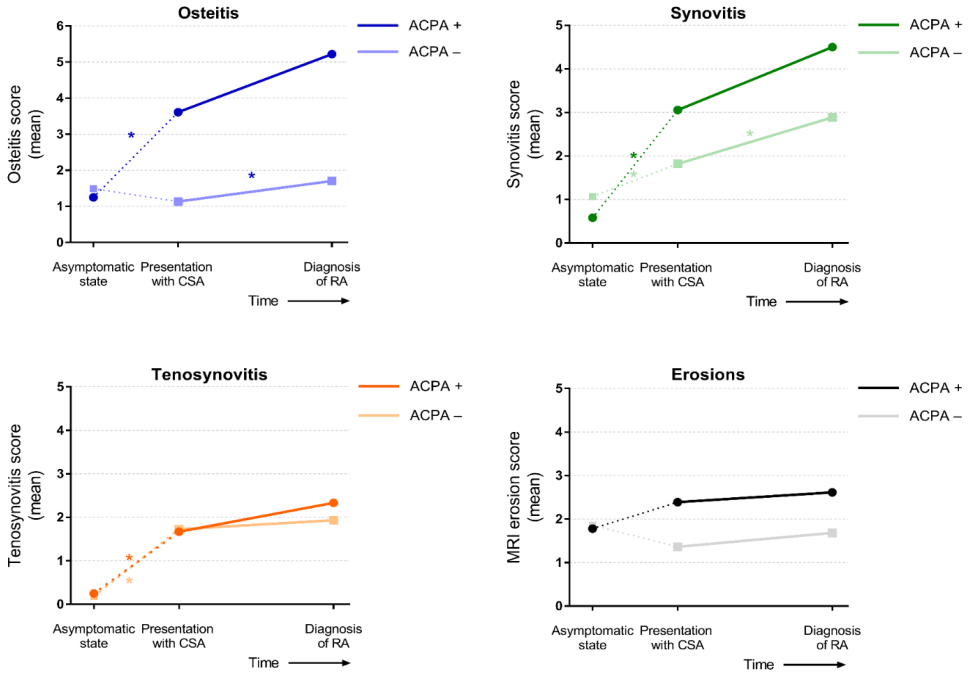
Patient characteristics of the different groups are provided in Supplementary Table 2.

Figure 2. MRI features of joint inflammation and erosions in patients that developed RA



Legend:
MRIs were performed at presentation with arthralgia and at diagnosis of RA. MRIs were not made in the asymptomatic phase but the course of local inflammation before presentation with arthralgia was estimated by comparisons with age-matched symptom-free persons (1:2 ratio to patients). Since these data were not measured longitudinally, data are presented in dashed lines. * indicates statistical significance at $p<0.05$ level.

Figure 3. MRI features of joint inflammation and erosions in patients that developed RA stratified for ACPA status



Legend:
MRIs were performed at presentation with arthralgia and at diagnosis of RA. MRIs were not made in the asymptomatic phase but the course of local inflammation before presentation with arthralgia was estimated by comparisons with age-matched symptom-free persons (1:2 ratio to patients). Since these data were not measured longitudinally, data are presented in dashed lines. * indicates statistical significance at $p<0.05$ level for a paired t-test.

Chapter 9

Improvement of symptoms in Clinically Suspect Arthralgia and resolution of subclinical joint inflammation — a longitudinal study in patients that did not progress to clinical arthritis

R.M. ten Brinck [1], D.M. Boeters [1], H.W. van Steenbergen [1], A.H.M. van der Helm–van Mil [1,2]

1. Department of Rheumatology, Leiden University Medical Centre, Leiden, the Netherlands
2. Department of Rheumatology, Erasmus Medical Centre, Rotterdam, The Netherlands

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Abstract

Introduction: Arthralgia and MRI-detected subclinical inflammation can precede development of clinically evident Rheumatoid Arthritis (RA). However, part of the patients presenting with Clinically Suspect Arthralgia (CSA) do not progress to RA. In these 'non-progressors', we aimed to study frequencies of spontaneous improvement of arthralgia, and its relation with the course of subclinical inflammation.

Methods: Between April 2012-April 2015, 241 patients were considered at risk for RA based on the clinical presentation and included in the CSA-cohort. 152 patients with complete data on clinical follow-up did not develop clinical arthritis, of which 98 underwent serial 1.5T MRI-scans (wrist, MCP2-5, and MTP1-5 joints) at baseline and after two-years. MRI-scans were scored for synovitis, tenosynovitis and bone marrow oedema (summed: MRI-inflammation score). MRI-scores were compared to scores of symptom-free persons.

Results: After two-year follow-up, 33% of the 'non-progressors' had complete resolution of symptoms; 67% had no symptom resolution and were diagnosed as: persistent CSA (44%), osteoarthritis (10%) and tendinomuscular complaints (13%). With symptom-free controls as reference, patients without resolution did not have increased MRI-scores at any time-point. However, patients achieving resolution of symptoms had increased MRI-inflammation scores at baseline (4.0 vs. 2.6, p=0.037), but not after two-years (3.0 vs 2.6; p=0.57) and during follow-up their MRI-inflammation score decreased significantly (p=0.036).

Conclusions: A subgroup of CSA-patients that did not progress to RA had spontaneous improvement of symptoms and resolution of subclinical joint inflammation. This time-relationship suggests that symptoms and inflammation were causally related in these patients. Further research is needed to identify mechanisms underlying resolution of inflammation.

Introduction

Rheumatoid arthritis (RA) can be preceded by a phase of preclinical disease with signs and symptoms, in which joint swelling cannot yet be identified through physical examination.[1] More than 90% of patients that develop RA had MRI-detected subclinical inflammation in small joints in the symptomatic phase of Clinically Suspect Arthralgia (CSA). However, of all patients that are identified as having CSA, a large part (up to 80%) do not progress to clinically evident RA.[1] Thus far, most longitudinal studies performed in patients considered at risk for RA focussed on progression from arthralgia to RA[1,2], since (early) identification of individuals that will develop RA is a key point from a clinician's perspective. However, there is also a group of patients that were considered at risk for RA but over time do not develop RA, meaning that in hindsight they possibly have not been truly 'pre-RA'. This subgroup of patients is unexplored and the course and outcome of joint symptoms and subclinical inflammation in these patients is yet unknown. From a clinical perspective, knowledge of the course of these symptoms could be useful. Moreover, despite non-progression, subclinical joint inflammation could be present in (part of) these patients at first presentation and comprehension on the natural course and severity of subclinical inflammation, and its relationship with spontaneous disappearance of arthralgia, increases our understanding on spontaneous resolution occurring in patients at risk phases of RA.

Longitudinal studies performed in the disease phase of early undifferentiated arthritis (UA) have shown that clinical synovitis resolved spontaneously in 10–40%, without intervention with disease-modifying antirheumatic drugs (DMARDs).[3,4] Based on these data, it can be hypothesized that a similar (or even larger) percentage of patients with CSA will show spontaneous resolution of joint symptoms. In addition, as arthralgia is associated with the presence of local subclinical inflammation[5], it could be hypothesized that there is a causal relation and that resolution of symptoms is connected to improvement of subclinical

inflammation presuming. Furthermore, it could be presumed that patients with persistent symptoms had more severe subclinical inflammation at presentation and during follow-up compared to patients with symptom resolution.

We aimed to increase understanding of the course of symptoms in patients that presented with CSA but did not progress to RA. Therefore the percentage of patients with symptom resolution and with persistent symptoms during two-year follow-up were determined. The scores of MRI-detected inflammation, and the time relationship with evanescence of symptoms, were studied. Finally MRI-data were compared to MRI-data obtained from age-matched symptom-free persons from the general population to estimate if MRI-detected joint inflammation returned to normal values.

Methods

Patients

Between April 2012-April 2015, 241 patients were included in the CSA cohort: CSA patients had no clinically evident arthritis, but recent-onset (<1 year) arthralgia of small joints that was clinically considered at risk for RA by the rheumatologist at first presentation at the outpatient clinic. The cohort has been described before in [6]. Routine follow-up visits were performed at 4, 12 and 24 months. If necessary (for instance when the patient experienced more symptoms or noticed a swollen joint) patients were seen in between scheduled visits by their rheumatologist. Hence, logistics were arranged such that patients in this cohort had very easy access to rheumatologic care; should a patient develop clinically evident IA, this was identified at the first opportunity. None of the patients were treated with DMARDs (including corticosteroids) during the course of the study. At the baseline visit, IgG ACPA (EliA CCP (anti-CCP2), Phadia, Nieuwegein, the Netherlands) and IgM RF (as described previously, in-house ELISA[7]) were determined. The cut-off for ACPA positivity was >7 U/ml, and for RF positivity it was >3.5 IU/ml.

A flowchart of inclusion is provided in Figure 1. As this study focused on patients that did not convert to RA over time, 45 patients that were diagnosed with RA during follow-up (clinical synovitis identified at physical examination by experienced rheumatologists, 19% out the total n=241) were excluded. From the subsequent total of 196 eligible patients, 44 patients were excluded because of inappropriate inclusion (n=5), or were lost to follow-up during the two-year course of the study (n=39). This resulted in complete clinical and follow-up data in 152 patients. Of these, 98 patients also had complete serial imaging data at two-year follow-up. Reasons for incomplete serial imaging were: contra-indications for contrast-enhanced MR-imaging or not willing to undergo (repeated) MR-imaging. Indications of potential selection bias at the different stages of the flowchart (n=241: all patients presenting with CSA, vs. n=196: eligible non-converting patients, vs. n=152: non-converting patients with complete follow-up data, vs. n=98: non-converting patients with complete follow-up data and serial imaging) were evaluated by comparing baseline characteristics between different patient groups. All patients provided written informed consent. Ethics approval was provided by the local medical ethical committee.

Assessment of symptom resolution

The main outcome was patient-reported resolution of symptoms. This was assessed at the routine follow-up visits by asking patients to answer a written question if they considered their symptoms completely resolved or not (by literally inquiring: "are your symptoms still present"; yes or no). Patients in whom initial presenting features were resolved, but with new joint symptoms were classified in the non-resolution group. Resolution of any related symptom (as judged by patients themselves) at the 24 months visit was used as definition for symptom resolution.

In addition to this main outcome, pain scores on a Visual Analogue Scale (VAS: scale 0–10) were collected to evaluate the robustness of the main outcome; the course in VAS pain was also studied. Furthermore, 68 tender

joint counts (68-TJC) were studied. After two years without conversion to clinical arthritis, patients were mostly referred back to their GP with a clinical conclusion, unless rheumatologist and/or patients felt that longer follow-up at the rheumatology outpatient clinic was required. The clinical diagnosis after 2-years was also studied.

Symptom-free persons

To make inferences on the presence and severity of MRI-detected subclinical inflammation as compared to the general population, MRI data from the 98 CSA-patients were matched to data of MRI-detected subclinical inflammation from symptom-free persons.[8] Matching was based on age in a 1:1 ratio, since age was previously proven to influence the severity of MRI-detected subclinical inflammation[9]. Since sex was previously demonstrated to have no effect on MRI-detected inflammation[8,10] matching was not performed on sex. The 98 symptom-free persons had no history of inflammatory rheumatic diseases, no joint symptoms during the last month and no evidence of synovitis at physical examination. The symptom-free persons were recruited from the general population, as described in [8].

MRI

Unilateral MRIs of wrist, MCP2–5, and MTP1–5 were performed at presentation with CSA (most painful or in case of equally severe symptoms the dominant side) and at two-year follow-up (when follow-up ended) of that same side. An ONI MSK Extreme 1.5T MRI scanner (GE Healthcare, Wisconsin, USA) was used, as described previously[1] and in the Supplementary Methods. Patients were instructed not to use NSAIDs 24 hours prior to MRI, with 22 patients reporting daily use of NSAIDs at baseline. MRIs were evaluated for bone marrow oedema (BME; range 0–72), synovitis (range 0–33) [11], and tenosynovitis (range 0–54) [12]. These three features were summed in the total MRI-inflammation score. Each MRI was scored by two readers, who belonged to a pool of four experienced

readers (all had interclass correlations ≥0.90, see Supplementary Table 1). The mean scores of two readers was studied. All readers were blinded to clinical data and the order in time. MRI data were never reported to the clinicians in any phase of the study. Additional information on the scoring method is provided in the Supplementary Methods.

Analyses

Unpaired t-tests were used to compare patients with symptom-free persons. For analyses over time, paired t-tests were used. To evaluate if MRI-inflammation scores changed over time, analyses using measures of MRI-detected subclinical inflammation were confined to patients with a baseline total MRI-inflammation score of >0, as a baseline score of 0 would not be able to further decrease. 82 patients (84%) had a baseline MRI with a total MRI-inflammation score >0 (Figure 1).

For consistency, total MRI-inflammation scores on group-level for the same 82 patients were compared to scores of age-matched symptom-free persons. Furthermore, a sub-analysis within autoantibody-positivity (ACPA- and/or RF-positive; 19% of patients) CSA-patients was applied. Finally, sensitivity analyses were performed on the patients meeting the EULAR definition of arthralgia suspicious for progression to RA with ≥3 points (n=63). [13] Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS; version 23.0). P-values <0.05 were considered significant. Total MRI-inflammation scores are reported as mean.

Results

Patient characteristics

The baseline characteristics of patients at the different stages in the flowchart (Figure 1) did not show relevant differences, as shown in Table 1. Baseline characteristics of the patients with complete clinical follow-up and

MRI data at baseline and at two-year follow-up (n=98) are demonstrated in Table 2. Patients presenting with CSA that did not progress to RA were female in 74%, had a mean age of 47 years, a median 68-TJC of 5 joints, and 19% carried RA-related autoantibodies (RF and/or ACPA). These characteristics are comparable with previous reports on patients from the Leiden CSA cohort[1,14], although the percentage of autoantibody-positive patients was lower in this study in non-progressors, since the presence of autoantibodies is a risk factor for progression to RA[1,14] and autoantibodies were thus less often observed in the non-converting patients. MRI-detected inflammation was not associated with increased C-reactive protein levels (p=0.38).

Resolution of symptoms over time

In the total group of 152 non-converting patients, 38% (57 patients) indicated to have resolution of symptoms after two years follow-up and 63% (95 patients) had no symptom resolution. Similarly, in the group of 98 patients with serial imaging, 33% of patients (n=32) reported resolution of symptoms whereas 67% (n=66 patients) did not. In addition, in the 54 patients without serial MRIs, 25 experienced symptom resolution (46%) whereas 29 patients did not experience resolution of symptoms (54%). A chi-squared test comparing the number of patients experiencing symptom resolution in the groups of patients with and without serial MRI showed no significant difference (p=0.09). The percentages of patients with complete clinical and imaging data indicating to experience resolution of symptoms (n=32) at the follow-up visits at 4, 12, and 24 months are indicated in Figure 2.

Within the patients that had complete clinical and MRI data, the patients that indicated to have resolution of symptoms had a larger decrease in VAS pain scores over time than patients without resolution of symptoms (decrease in VAS pain of 2.9 versus 0.77; $p<0.001$; Figure 3). At baseline, the median 68-TJC was 4 in patients with resolution and 6 in patients without resolution. After 2 years the median TJC was 0 in the patients with

symptom resolution, while this was significantly higher in the patients without symptom resolution (Mann-Whitney U test: p=0.02). Several other characteristics of both groups evaluated at two-year follow-up are presented in Table 3. Although resolution of symptoms was initially assessed with one question, these results show that patients that reported to have symptom resolution improved in other measures for pain.

Patients with remaining symptoms were diagnosed as: persistent CSA because of persistent inflammatory type of arthralgia according to the rheumatologists (n=43; 44% of all non-converters), osteoarthritis (n=10; 10% of all non-converters) and tendinomuscular complaints (n=13; 13% of all non-converters).

At disease presentation, the proportion of patients that used NSAIDs on a daily basis was equally distributed between patients with or without resolution of symptoms (22% versus 23%; p=0.89). After two-year follow-up, 9% of the patients with persistent symptoms used NSAIDs on a daily basis, whilst NSAIDs were not used in the group with symptom resolution, which is in line with absence of symptoms.

Clinical characteristics of patients with and without symptom resolution

Patients that later-on achieved symptom resolution had no differences in baseline characteristics at baseline; Table 2 displays the patient characteristics for the 152 non-converting patients with complete clinical follow-up data, as well as the 98 non-converting patients with serial MRIs. The mean baseline total MRI-inflammation score was slightly higher in patients that would eventually achieve symptom resolution (3.5) as compared to patients with persistent symptoms (2.7), but this was not statistically significant (p=0.33).

Association between symptom resolution and improvement of MRI inflammation

The mean total MRI-inflammation scores of the 82 patients with a baseline total MRI-inflammation score >0 were compared to MRI-scores of similar age-matched symptom-free persons to infer if the MRI-inflammation scores at the different time points exceeded the level of MRI-detected inflammation prevalent in the general population. Other characteristics of the symptom-free persons are provided in Supplementary Table 2.

In the group of CSA patients that achieved resolution of symptoms over time, the mean MRI-inflammation score was higher than that of symptom-free persons at baseline (4.0 vs 2.6; p=0.04; Figure 4). In contrast, the patients that did not report resolution of symptoms did not have higher MRI inflammation scores at baseline (mean 3.3 and 2.9 respectively; p=0.26; Figure 4).

After two-year follow-up, the mean total MRI-inflammation score in patients with resolution of symptoms decreased to a level similar to that of symptom-free persons (3.0 vs 2.6; p=0.57; Figure 4), whereas the patients without resolution of symptoms still had no differences in their total MRI-inflammation scores (mean 2.7 vs 2.9; p=0.68; Figure 4). Comparison of the individual inflammatory features as detected by MRI are provided in Supplementary Figure 1; the decrease in total MRI-inflammation-score was mostly due to decrease in tenosynovitis and synovitis.

Finally, the difference of the total MRI-inflammation scores over time were evaluated between baseline and two-year follow-up (Figure 4). The CSA patients with resolution of symptoms had a statistically significant decrease in MRI-inflammation score (difference 0.98; paired t-test: p=0.036). In the CSA patients that did not convert to RA and had no resolution of symptoms the decrease was smaller (difference 0.44) and did not reach statistical significance (paired t-test: p=0.09).

Together, in patients with resolution of symptoms, MRI inflammation scores were increased at first presentation and normalized after symptom resolution, whereas patients that remained having symptoms (but did not progress to RA) did not have increased inflammation scores at any time point, with age-matched controls as reference.

Although the group of patients without resolution of symptoms was a heterogenous group in terms of final diagnosis, none of the separate diagnoses had a significant difference in MRI-inflammation score over time: persistent CSA (p=0.37), osteoarthritis (p=0.60), and tendinomuscular complaints (p=0.79). Separate matching of the patients with persistent CSA compared to symptom-free persons revealed no differences in total MRI-inflammation score at baseline (3.4 vs 2.8; p=0.25), or at two-year follow-up (2.6 vs 2.8; p=0.83). Matching of patients finally diagnosed with osteoarthritis and tendinomuscular complaints with symptom-free persons was not performed due to small patient numbers.

Sub-analyses: autoantibody-positive patients

Although the presence of autoantibodies in CSA is associated with an increased risk on RA development, part of the patients with autoantibodies did not progress. In line with previous studies that reported a PPV of >60% for ACPA-positive patients[1,14], part of the autoantibody-positive patients did not progress to RA during two-year follow up. In our data 19% of the non-converting patients were either ACPA- or RF-positive. There was no conversion in ACPA- or RF-status in any direction over 2 years' time.

Within the group of ACPA- or RF-positive non-converting patients (n=19), 7 patients (37%) had symptom resolution over time and 12 patients (63%) had no resolution of symptom. The total MRI-inflammation score decreased from 5.0 to 3.3 (difference 1.8; paired t-test: p=0.21) in the patients with resolution of symptoms. In the patients without resolution of complaints, the total MRI-inflammation score reduced from 2.4 to 1.9 (difference 0.55;

paired t-test: p=0.19). Comparison of MRI-scores with symptom-free persons, as stratified by resolution of symptoms, was not performed due to insufficient statistical power.

Sensitivity analysis: patients meeting the EULAR definition

A sub-analysis was performed in the patients that met the EULAR definition of arthralgia suspicious for progression to RA.[13] 64% of the CSA patients that did not develop RA fulfilled the EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis with ≥3 items present. Also in this subgroup, 37% of the patients achieved spontaneous resolution of symptoms.

Similar findings were obtained when patients meeting the EULAR definition and with a baseline total MRI-inflammation score >0 were compared to MRI-scores of similar age-matched symptom-free persons. The patients experiencing resolution had higher MRI-inflammation scores at disease presentation than symptom-free controls (p=0.04), while the scores were no longer increased at the time of symptom resolution (p=0.53). Patients without resolution of symptoms (that did not progress to RA) did not have significantly increased MRI-inflammation scores at any time point (Supplementary Table 3). Over time, MRI-inflammation scores decreased in the patients with symptom resolution, (4.6 to 3.1; p=0.02). In patients without symptom resolution, scores did not decrease: 3.3 to 3.2; p=0.67.

Discussion

Patients with Clinically Suspect Arthralgia are considered to be at risk for RA development by their rheumatologists. Most research done in the field of "RA risk" is focussed on the subgroup of patients that indeed progress to RA. However, a large proportion of the patients that are considered to have an increased risk do not actually develop IA and RA. Here, we studied

the group of non-converting patients and observed various outcomes. A considerable part of the patients that initially had presented with CSA continued to be characterized as CSA after two-year follow-up. A smaller part of the patients developed other explanations for their complaints. Interestingly, both latter groups of patients did not have increased MRI-inflammation scores of small joints as compared to age-matched symptom-free persons. Furthermore, approximately one-third of the non-converting patients had resolution of symptoms over time. These patients had increased MRI-detected subclinical inflammation at baseline, which also resolved over time. This time relationship suggests that the subclinical inflammation was related to the presence of symptoms and the phenotype of CSA. In our view, this is the most interesting group of patients: these patients may indeed have been pre-RA but one of several final switches required for actual progression to RA were not turned "on" and the disease process resolved without intervention.

Our study is the first to quantify the percentage of patients presenting with CSA that will have resolution of symptoms over time. It consists of one-third of all non-progressing patients and 27% of all patients that were identified as having CSA by rheumatologists. Interestingly, previous studies done in patients with UA showed that clinical synovitis resolved in 10–40%[3,4], which is a similar range of spontaneous dissolvement. Similar as seen here, patients with spontaneous resolution were more often autoantibody-negative than patients with progression to arthritis. Despite the association with the absence of autoantibodies, the pathophysiologic mechanisms mediating spontaneous resolution or absence of progression are not elucidated yet. Our study served to identify this group of patients. Future studies are required to increase our understanding on the biological mechanisms involved.

This study had several limitations. First, patients were analysed during two-year follow-up and patients that did not progress to RA could still

develop IA after the follow-up of the study ended. However, as the Leiden University Medical Centre is the only referral centre in the region, it is unlikely that patients will visit another centre should symptoms reoccur. This allowed us to study if patients had returned to our Rheumatology department after the formal final regular follow-up visit at two years. After an average of 5 years after the baseline visit, none of the patients had returned to be diagnosed with RA, indicating that patients truly did not develop RA. In addition, patients that had indicated that symptoms had disappeared after two years could theoretically experience renewed symptoms later-on in life. However, this would not affect the current findings that resolution of symptoms was paralleled by resolution of subclinical inflammation.

A further limitation of our study is the small number of patients included. Especially the number of patients that was ACPA-positive and not progressed to RA is small, which warrants future studies with larger numbers of included patients to allow statistically more powerful analyses than our current, mostly exploratory, analyses.

Another limitation is that part of the patients did not complete follow-up, or did not consent to undergo another MRI. Although missing data was presumably not at random as patients with less severe symptoms are more likely to retract from follow-up, the patient characteristics of the different groups were quite similar (Table 1), arguing against a major bias. However, the percentage of patients experiencing symptom resolution in the group that did not have complete imaging data over two years was slightly larger (46%) than the percentage of patients with complete imaging data (33%) which could be a potential source of bias, although the difference was not significantly different. Possibly patients who experienced symptom resolution slightly less often felt the need to undergo imaging studies after two years.

Finally, since patients with baseline MRI-scores of >0 were studied, regression to the mean could have occurred. Furthermore, scores of MRI-detected inflammation were studied on a group-level rather than joint-level to decrease the possibility of type 1 error due to multiple testing. Nevertheless, we demonstrated that baseline scores in the patients with resolution significantly exceeded the level of MRI-detected subclinical inflammation of symptom-free persons, but not in the patients without resolution of symptoms.

The main outcome was patient-reported resolution of symptoms. No validated questionnaire exists of patients with arthralgia at risk for RA and we assessed this outcome using a single written question. The robustness of this outcome was illustrated by decreasing VAS pain scores and diminishing tender joint counts in the patients with resolution and therefore we considered this to be a valid question that was interpreted well and uniformly by patients themselves.

Finally, DMARD therapy (including steroids) was not allowed and not prescribed during the course of the CSA study, but NSAIDs were allowed. NSAIDs were stopped before MR-imaging. It could be questioned if NSAIDs played a role in disease resolution. However, NSAIDs are generally not considered as disease-modifying therapy, and the frequency of NSAIDs use at baseline was similar in the patients with and without symptom resolution.

In conclusion, one-third of all patients with CSA that did not convert to IA or RA during two-year follow-up had resolution of symptoms and improvement of subclinical joint inflammation. This time relationship is suggestive for a causal relation of the subclinical inflammation and the phenotypic presentation of CSA. Further research is needed to identify mechanisms that are involved in resolution of disease processes.

Supplementary information

Supplementary data is available at the website of Arthritis Research & Therapy, or can be obtained by contacting the first author.

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Table 1. Comparison of baseline patient characteristics between different stages of the flowchart as presented in Figure 1.

Patient characteristic	N=241	N=196	N=152	N=98
Age in years, mean (SD)	44 (13)	44 (13)	45 (13)	47 (13)
Female sex, N (%)	187 (78)	152 (77)	118 (78)	73 (74)
Family history of RA, N (%)	71 (30)	52 (27)	43 (28)	28 (29)
Symptom duration in weeks, median (IQR)	18 (10 – 48)	17 (9 – 30)	17 (9 – 33)	17 (9 – 43)
Presence of morning stiffness ≥60 minutes, N (%)	80 (33)	61 (35)	49 (32)	29 (30)
68-TJC, median (IQR)	6 (3 – 10)	6 (2 – 11)	6 (2 – 10)	5 (2 – 10)
VAS pain score, median (IQR)	5 (3 – 7)	5 (3 – 7)	5 (3 – 7)	5 (3 – 6)
≥3 items on EULAR definition of arthralgia suspicious for progression to RA[13], N (%)	178 (74)	141 (72)	100 (66)	63 (64)
Increased CRP (≥5 mg/L), N (%)	53 (22)	39 (20)	29 (19)	19 (19)
Autoantibody status				
Negative for IgM-RF and ACPA, N (%)	184 (76)	166 (84)	125 (82)	79 (81)
ACPA- or RF-positive, N (%)	57 (24)	31 (16)	27 (18)	19 (19)

Legend:
ACPA = anti-citrullinated peptide antibody (positive if: ≥7 U/mL); CRP = C-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor (positive if: ≥3.5 IU/mL); IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; TJC = tender joint count.

Table 2. Baseline characteristics of the Clinically Suspect Arthralgia patients with complete clinical follow-up (N=152) and complete clinical follow-up as well as MRI data at baseline at two-year follow-up (N=98)

Patient characteristic	Complete clinical follow-up (N=152)		Complete clinical follow-up and MRI data (N=98)	
	Symptom resolution (n=57)	No symptom resolution (n=95)	Symptom resolution (n=32)	No symptom resolution (n=66)
Age in years, mean (SD)	44 (13)	46 (13)	46 (14)	47 (13)
Female sex, N (%)	40 (70)	79 (82)	20 (63)	53 (80)
Family history of RA, N (%)	17 (30)	26 (27)	10 (31)	18 (27)
Symptom duration in weeks*, median (IQR)	17 (9 – 30)	17 (9 – 41)	18 (15 – 32)	17 (9 – 50)
Morning stiffness ≥60 minutes, N (%)	22 (39)	27 (28)	10 (31)	19 (29)
68-TJC*, median (IQR)	5 (2 – 8)	6 (2 – 12)	4 (2 – 7)	6 (2 – 13)
≥4 tender joints, N (%)	33 (58)	61 (64)	18 (56)	43 (65)
Increased CRP (≥5 mg/L), N (%)	12 (21)	17 (18)	9 (28)	10 (15)
Autoantibody status				
Negative for IgM-RF and ACPA, N (%)	43 (75)	71 (75)	25 (78)	54 (82)
ACPA- or RF-positive, N (%)	9 (16)	18 (19)	7 (22)	12 (18)
ACPA-positive, N (%)	5 (9)	6 (6)	3 (9)	4 (6)

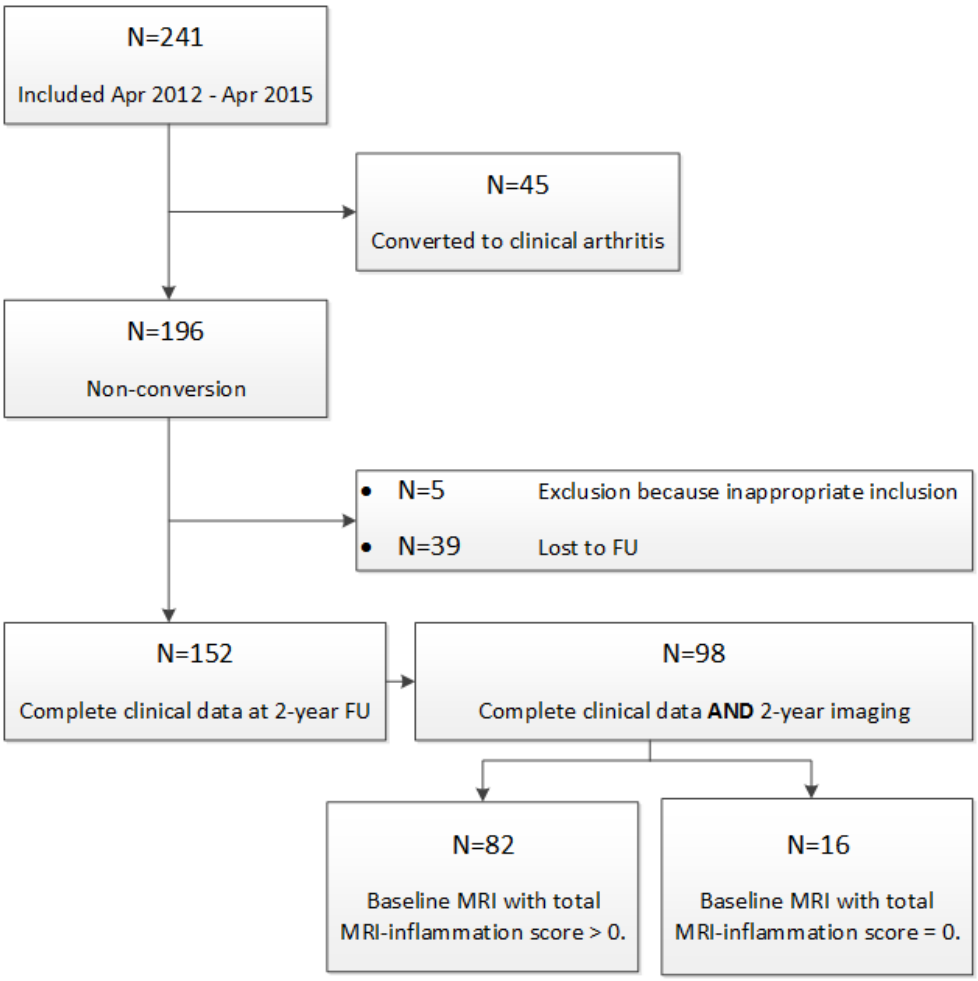
Legend:
ACPA = anti-citrullinated peptide antibody (positive if: ≥7 U/mL); CRP = C-reactive protein; IgM-RF = immunoglobulin M rheumatoid factor (positive if: ≥3.5 IU/mL); IQR = interquartile range; RA = rheumatoid arthritis; SD = standard deviation; TJC = tender joint count; VAS = visual analogue scale. *Missing data were as follows: symptom duration in weeks (n=4), 68-TJC (n=1).

Table 3. Characteristics of the 98 patients with and without resolution of symptoms at two-year follow-up

Patient characteristic	Symptom resolution (n=32)		No symptom resolution (n=66)		P-value
68-TJC, median (IQR)	0	(0 – 0)	1	(0 – 4)	0.02
Presence of morning stiffness ≥60 minutes, N (%)	5	(16)	14	(21)	0.56
HAQ-score, mean (SD)	0.18	(0.40)	0.60	(0.50)	0.09
VAS pain score, mean (SD)	0.87	(1.5)	4.2	(2.4)	<0.001
VAS fatigue score, mean (SD)	3.7	(3.3)	5.6	(2.6)	0.003

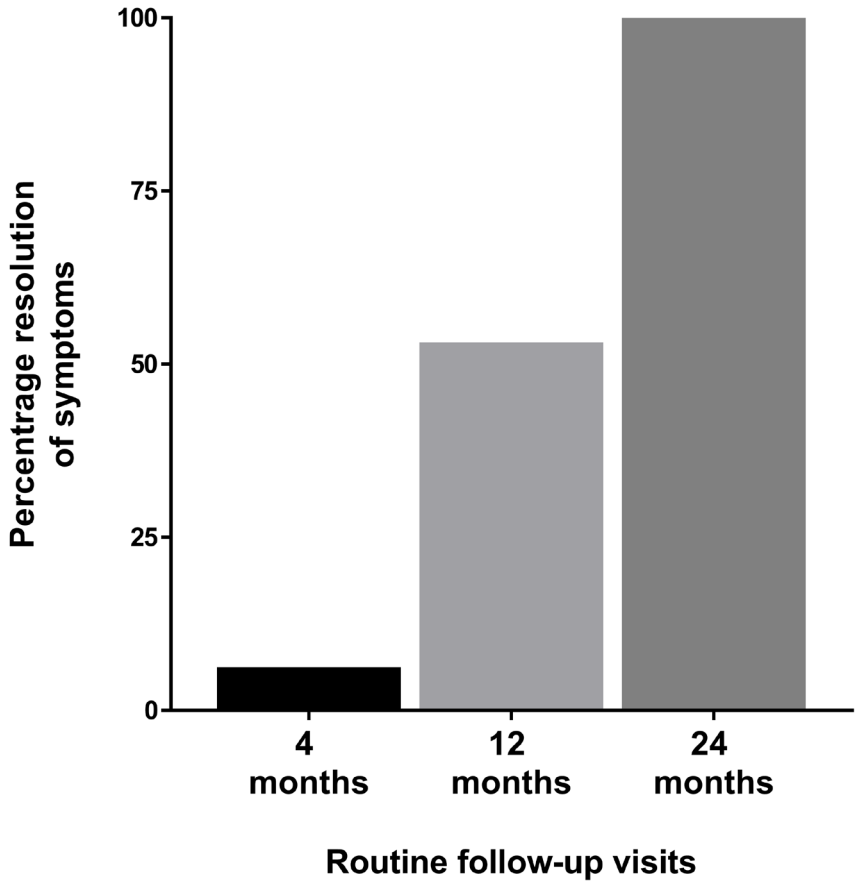
Legend:
IQR = interquartile range; SD = standard deviation; TJC = tender joint count; VAS = visual analogue scale, range 0-10.

Figure 1. Flowchart of the different patient populations



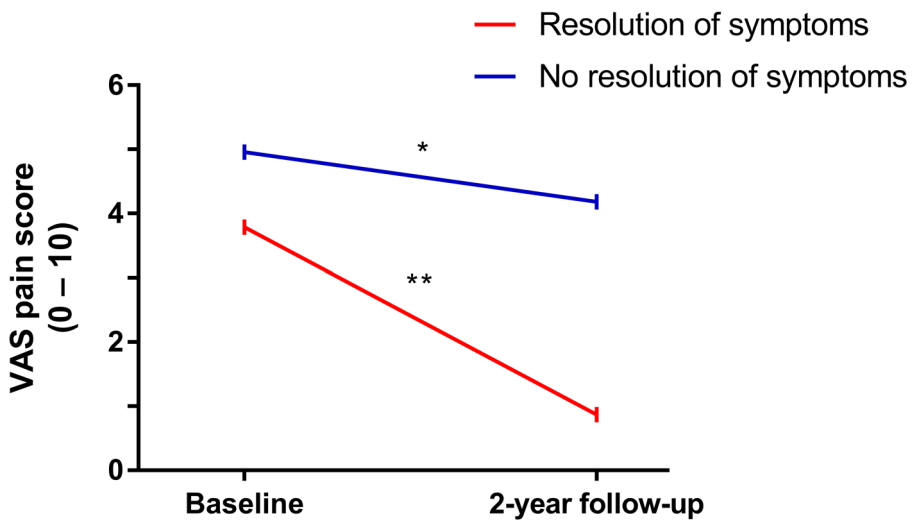
Legend:
FU = follow-up.

Figure 2. Percentage of patients reporting resolution of symptoms per follow-up visit presented for all patients (N=32) that had resolution of symptoms



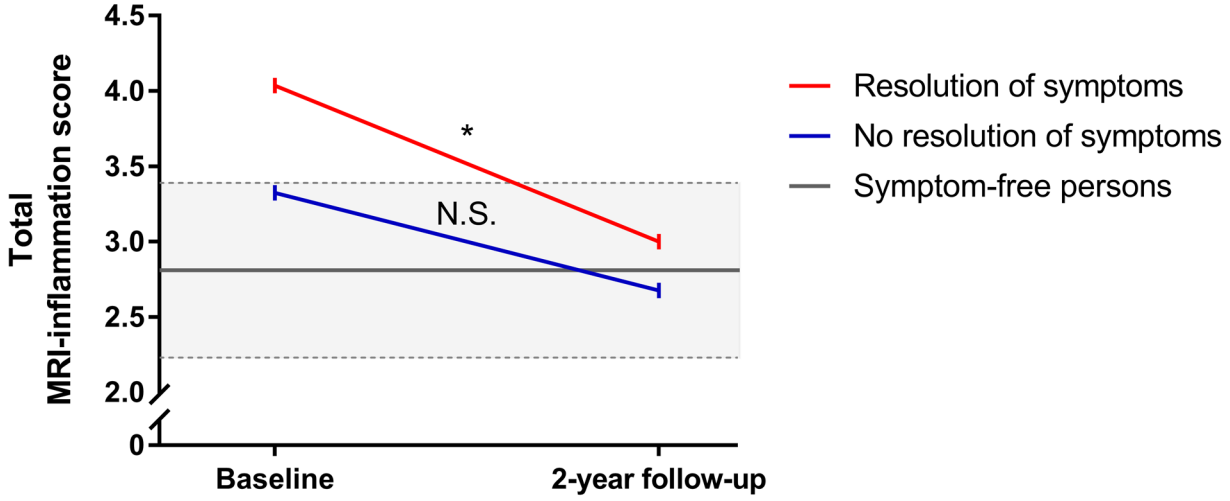
Legend:
Percentage of patients reporting resolution of symptoms per regular follow-up visit at 4, 12 and 24 months.

Figure 3. VAS pain scores over time for patients with and without resolution of symptoms (N=98).



Legend:
* indicates significance at the P<0.05 level, ** indicates significance at the P<0.01 level.

Figure 4. Mean total MRI-inflammation scores over time for patients with and without resolution of symptoms (N=82).



Legend:
The grey area indicates the mean and 95% confidence interval (dashed lines specify the upper and lower limit of the interval) of the total MRI-inflammation score in age-matched symptom-free persons. Considered in this Figure are patients with a baseline total MRI-inflammation score >0. At baseline, in the group without resolution of complaints the mean total MRI-inflammation score was not different as compared to symptoms-free persons (p=0.26). Patients with resolution did have higher scores than symptoms-free persons (p=0.04). After two-year follow-up, patients without and with resolution of symptoms both did not have higher scores (p=0.68 and p=0.57, respectively). * indicates significance at the P<0.01 level, N.S. indicates non-significance.

Chapter 10

Summary and future perspectives

Summary

This thesis focused on investigating the early identification of Rheumatoid Arthritis (RA), assessing the burden of disease, and enhancing understanding of disease mechanisms in the earliest disease phases. Many of the studies in this thesis focused on data from the Leiden Clinically Suspect Arthralgia (CSA) cohort. The CSA cohort is an inception cohort at the rheumatology outpatient clinic of the Leiden University Medical Centre, in Leiden, the Netherlands. CSA patients had recent-onset (<1 year) arthralgia of hand (MCP, PIP, wrist) or feet (MTP) joints, and they were considered at risk for RA, based on the clinical expertise of the rheumatologists.

Part 1: Predicting progression to RA

In the first part of this thesis, **chapters 2, 3 and 4** focused on the identification of RA and progression to RA in its earliest phases. **Chapter 5** evaluated the burden of disease during progression to RA.

Despite acknowledging recommendations in national and international guidelines on prompt referral of patients presenting with possible inflammatory arthritis (IA), general practitioners (GPs) indicate to feel uncertain in their proficiency to detect synovitis through joint examination.[1] Considering the need of early identification of RA, our objective in **chapter 2** was to develop and validate a rule composed of clinical characteristics to assist GPs and other physicians in identifying inflammatory arthritis (IA) when in doubt.

This was investigated in the Leiden Early Arthritis *Recognition* Clinic (EA*R*C); a screening clinic for patients in whom GPs suspected – but were unsure of – the presence of IA. In this cross-sectional study, associations with IA were found for: male gender, age ≥60 years, symptom duration

<6 weeks, morning stiffness >60 minutes, a low number of painful joints (1–3 joints), presence of patient-reported joint swelling, and difficulty with making a fist. In order to derive a tool that is easy to use in routine clinical practice in primary care, a simplified model was generated by rounding the obtained β's from the multivariable regression model to the nearest 0.5. This resulted in a simplified rule consisting of seven items and a total score ranging from 0 to 7.5 with corresponding predicted risks of IA. This rule showed reasonable discriminative ability for IA and could assist physicians in decision-making in patients with suspected IA, increasing appropriateness of health care utilization.

Furthermore, a simulation was run for the rule with a prevalence of inflammatory arthritis set at 20%. This simulation was performed to mimic applicability in primary care settings, as this setting will likely have a lower prevalence of IA than the EA*R*C-setting (which was situated in between primary and secondary care). Our simplified rule was implemented in a web application that provides probabilities on the presence of current IA for individual patients that can be accessed online. The CARE tool presented in **chapter 2** is different from other studies that derived tools to facilitate triage of patients that have been referred to secondary or tertiary care.[2–4] The CARE tool did not aim to prioritize patients that have been referred already, but rather assist in the decision-making and referral process. The tool aims to facilitate recognition of IA (as this would necessitate prompt referral to a rheumatologist) rather than forming a longitudinal study to predict development of a specific diagnosis (e.g. RA) later-on.

Chapter 3 assessed the risks for arthritis development of three different autoantibodies: ACPA, RF and/or anti-carbamylated protein antibodies (anti-CarP). ACPA and RF are already tested routinely in current clinical practice. Anti-CarP is a novel autoantibody that has gained popularity over the last few years.[5] Assessed separately, associations for arthritis development were observed for both ACPA, RF and anti-CarP

antibodies. However, correcting for simultaneous presence of the three autoantibodies revealed that only ACPA was significantly associated with arthritis development. The positive predictive values (PPVs) for arthritis development within 2 years were: 63% for ACPA, 53% for RF and 50% for anti-CarP antibodies. Concurrent presence of ACPA and RF yielded a PPV of 67%. Although the serum levels of ACPA were not significantly associated with increased progression to clinical arthritis, this was the case for RF levels. Our data implicates that ACPA confers the highest risk for arthritis development and is of additive value to RF. However, clinicians and researchers alike should be aware that this implicates that >30% of ACPA-positive/RF-positive CSA patients did not develop arthritis despite having arthralgia and positive autoantibodies. Thus, both CSA and information on autoantibodies are still insufficient for accurately identifying imminent autoantibody-positive RA.

It is noteworthy that PPVs are dependent on enrichment (i.e. prevalence) of cases in cohort studies, meaning that the same test may yield different results depending on the setting. Patients that are identified as having CSA by rheumatologists comprise a small group of all patients presenting with arthralgia to secondary care (<6%).[6] As a consequence, the CSA-patients evaluated in **chapter 3** likely had higher prior chances to develop RA than patients with non-specific arthralgia in secondary or primary care. In **chapter 4**, the implications of screening for two or three autoantibodies in persons at risk for RA was investigated. We found no additional predictive value was found for testing an autoantibody additive to ACPA and RF.

In **chapter 5,** we assessed functional disability in patients with CSA. Although several studies have focused on biomarkers to identify patients in the arthralgia phase preceding RA, the disease burden of this phase for patients is less studied. We found that scores of functional disability on the Health Assessment Questionnaire Disability Index (HAQ-DI) were already elevated during the phase of CSA: 0.50 for all patients. This was in line with HAQ-DI scores from ACPA-positive arthralgia patients from another

study.[7] A score on the HAQ-DI of 0 indicates no difficulty, whereas a score of 1 represents some difficulty. Furthermore, we established that higher MRI-inflammation scores were associated with higher functional disability. Interestingly, during follow-up, increased scores of functional disability (≥1.0) were associated with subsequent development of arthritis. Previous studies in early RA cohorts have shown that mean HAQ-scores at presentation were also 1.0.[8,9] Moreover, within patients converting to RA, scores of functional disability did not increase from presentation with CSA to arthritis development (0.88 and 0.75, respectively). Together, these data show that functional limitations in the symptomatic pre-arthritis phase of CSA were as serious as in the early clinical phase or RA, with the maximal level of disability in this phases of disease already present when presenting with CSA. These findings illustrate the relevance of the arthralgia phase from a patients' perspective.

Thus, in conclusion of the first part of this thesis, the early recognition of RA could (possibly) be further enhanced by the use of the CARE tool, perchance with the addition of other clinical parameters and autoantibodies. Nevertheless, there remains a large proportion of patients that cannot be accurately identified despite a suspect pattern of signs and symptoms, as well as information on autoantibodies.

Part 2: Disease mechanisms involved in progression from CSA to RA

The second part of this aimed to provide insights into the processes involved in development of RA by understanding the biological mechanisms (**chapter 6**) and longitudinal investigations using MRI to study inflammation at joint level (**chapter 7**), and at the patient level (**chapter 8**) over time. In **chapter 8**, the longitudinal diagnoses as well as their mutual time relationship with MRI-detected subclinical inflammation were scrutinized.

ACPAs are hypothesized to directly activate osteoclasts, which lead to bone erosions and pain in RA. In **chapter 6,** evidence was sought for this hypothesis in humans. It evaluated whether in patients with arthralgia who are at risk of RA, ACPA is associated with erosions as detected by MRI, independent of inflammation, and also independent of the presence of rheumatoid factor (RF).

Indeed, ACPA-positive patients had higher erosion scores than ACPA-negative patients. Nevertheless, this mechanism was likely mediated by concurrent presence of inflammation: ACPA-positive patients without concomitant subclinical inflammation did not have higher erosion scores than ACPA-negative patients. This was in contrast to ACPA-positive patients with local inflammation, whom had higher erosion scores than ACPA-negative patients. Mediation analyses suggested that local inflammation lies in the causal path of ACPA leading to higher erosion scores. Stratification was also applied for ACPA and RF: compared to ACPA-negative/RF-negative patients, ACPA-positive/RF-negative patients did not differ in terms of erosions as detected by MRI, but ACPA-positive/RF-positive patients did have higher erosion scores. Finally, triple stratification for ACPA, RF and inflammation demonstrated that the presence of ACPA and/or RF is only associated with higher erosion scores if concomitant inflammation is present.

It is intriguing to speculate how –or if– ACPAs contribute to joint erosions together with inflammation. However, since **chapter 6** was an association study, it does not allow conclusions on biological mechanisms. The results align with those obtained in patients with early rheumatoid arthritis[10,11] and fuel the hypothesis that ACPAs alone are not the main and/or single pathogenic factor contributing to joint erosions and consequent destruction. In sum, the effect of ACPA on erosions is mediated by inflammation and is not independent of RF.

Within patients that develop IA, the course of inflammation at joint level during the transition from CSA to RA is unknown. **Chapter 7** assessed progression of MRI-detected subclinical inflammation at the joint level. It was investigated if development of clinical arthritis as imaged fitted better with the idea that arthritis development is a local outgrowing process, or whether the data are more in line with the hypothesis of a systemic deregulation; in this case locations of subclinical inflammation and synovitis development were largely uncoupled.

350 joints of 35 patients presenting with CSA (with all patients subsequently developing clinical arthritis) were studied at presentation with CSA and subsequently when clinical synovitis was first identified at physical examination. At presentation with CSA, 71 joints showed subclinical inflammation. During progression to IA, 20% of these joints had resolution of inflammation, 60% had persistent inflammation and 20% progressed to clinical synovitis. Of all joints that had developed clinical synovitis, no prior subclinical inflammation was detected in 69%. The present joint-level observations on inflammatory progression fit best with the hypothesis of 'global deregulation', rather than that of a localized exacerbating process. Previous observations of increased markers of systemic inflammation in pre-arthritis phases may support this.[12]

As only moderate correlations were demonstrated in **chapter 7** between joints with subclinical inflammation and joints that developed clinical synovitis, our data implies that development of RA is a more systemic, rather than a locally outgrowing process.

Like in chapter 7, the study in **chapter 8** assessed the course of MRI-detected subclinical joint inflammation during progression to RA, only now on a patient level. Several hypotheses contrast each other on this course. Firstly, it has been postulated that synovitis is an initial process that is succeeded by bone involvement ('outside-in hypothesis').[13–16] Alternatively, it has been suggested that RA is a primary bone marrow disease, which subsequently encroaches upon the synovial membrane

('inside-out hypothesis').[14,17,18] Lastly, it has been hypothesized that these processes could emerge and progress simultaneously.[14,19]

To address this outstanding question, patients were studied that progressed from CSA to RA. At presentation with CSA, synovitis and tenosynovitis scores were higher than scores observed in age-matched symptom-free persons, which suggests that these had already increased in the phase preceding first presentation with arthralgia. At first presentation, ACPA-positive arthralgia patients also had increased osteitis scores. During progression to RA, synovitis and osteitis increased significantly, in contrast to tenosynovitis and erosion scores. This pattern was again similar in both ACPA-subsets, although statistical significance was reached for synovitis and osteitis in ACPA-negative, but not in ACPA-positive RA. The increased tenosynovitis and synovitis-scores at CSA-onset and the increase in synovitis and osteitis during progression to RA suggest an 'outside-in' temporal relationship of arthritis development; particularly for ACPA-negative RA. For ACPA-positive RA further studies are needed.

As synovitis and osteitis mostly increased during the symptomatic pre-arthritis phase, our data implies that inflammation mainly starts outside the bone (fitting the 'outside-in hypothesis'). Only later on (after presentation with CSA) does osteitis increase. Presence of BME then poses the joint at risk for structural damage development in the phase of clinically evident arthritis (if left insufficiently treated).[20] It needs to be noted that absolute patient number were rather small in **chapter 8**. The group of ACPA-positive RA patients comprised only 9 persons and therefore the statistical power to detect an effect was likely low.

Finally, the outcomes in CSA patients not progressing to RA are unknown. **Chapter 9** determined the course of joint symptoms and mutual time relationships with MRI-detected subclinical inflammation. After two years follow-up, one-third of patients that did not convert to clinical arthritis had complete resolution of symptoms; whereas two-third of patients had

persistent symptoms. Interestingly, in all patients presenting with CSA, the progression rate to RA is approximately 20%.[21] Consequently, of the 80% of patients not converting to RA, two-third did not experience resolution of complaints (53% of all CSA), whereas one-third of the patients in this study had resolution of symptoms (27% of all CSA). Studies in UA patients showed that clinical synovitis will spontaneously resolve in 10-40% of patients[22,23], which is a similar range of spontaneous dissolvement of symptoms as in CSA.

Next, total MRI-inflammation scores (sum of bone marrow edema, synovitis and tenosynovitis) were compared to symptom-free persons for both patients with resolution of symptoms and patients with persistent symptoms. In the group with persistent complaints, scores were not different at baseline and after two years follow-up compared to symptom-free persons. Contrarily, patients with resolution had elevated scores at baseline, but no longer at two-year follow-up; which would suggest dissolvement of MRI-detected subclinical inflammation over time. This mutual time relationship may suggests that subclinical inflammation was causally related to arthralgia. Taken together, our data showed that spontaneous resolution is possible in the phase of CSA and that patients presenting with CSA that had spontaneous resolution of symptoms also had dissolvement of subclinical inflammation.

All studies in this part analysed disease mechanisms and MRI-detected subclinical inflammation in the development of RA. Interestingly, resolution of symptoms and subclinical inflammation (as detected by MRI) was possible in CSA-patients. Furthermore, the studies in patients converting to RA suggested that progression of clinical arthritis is a systemic inflammatory process that follows an 'outside-in' temporal relationship in which synovitis is an initial process that is succeeded by bone involvement.

Future perspectives and conclusions

In recent decennia, major advances have been booked in the early identification and treatment of patients with RA. These advances, in combination with improved treatment strategies, have dramatically improved the long-term outcomes. Four decades ago, the treatment of RA consisted of a combination of resting, physiotherapy and analgesics. Patients and physicians accepted debilitating deformities due to structural articular bone damage. Patients would often enter the physician's consulting room using a wheelchair. Recently however, diagnostic workup and treatment of RA patients has improved so considerably that wheelchairs are hardly present anymore in the waiting room of a rheumatology outpatient clinic. Structural deformities are becoming less and less prevalent because disease activity can be reasonably well-controlled.

One of the keys to this advancement lies in the early identification of RA. The last decade has been a rapid current of important discoveries in early RA. Although it is still a novel and relatively young area of research, in Clinically Suspect Arthralgia (CSA) the rheumatologists will rely on pattern recognition of signs and symptoms to recognize RA before clinically swollen joints can be identified at physical examination. A combination of arthralgia in the small joints of hand and feet, functional limitations with morning stiffness might suggest the development of RA.

Several strategies for early identification exist, one of which is the aforementioned approach of CSA. Recognition of CSA requires the clinical expertise of a rheumatologist, and general practitioners (GPs) might be lacking this expertise due to the low incidence of patients with actual inflammatory arthritis despite a large numbers of presentations with musculoskeletal symptoms. Considering the need for accurate prediction models to estimate which patients will progress to RA over time, we

developed the CARE tool in **chapter 2.** External validation of the results presented in chapter 2 is required.

First of all, because a setting in between primary and secondary (the Early Arthritis *Recognition* Clinic) was used, its true discriminative ability for IA needs to be confirmed when actually used in a primary care setting. These validation studies in actual primary care setting will have to demonstrate if the CARE tool can truly function as an addition to current diagnostic workup by GPs.

As mentioned in **chapter 2**, the addition of other (clinical) variables might increase the discriminative ability for RA and may be a subject of future studies. Examples of additional variables include the "squeeze test" of MCP joints, or imaging abnormalities. The value (and possible addition or exclusion) of each variable in the rule needs to be analyzed in primary care setting.

Furthermore, GPs in the Netherlands function as a strict gatekeeper for physicians in secondary care, such as rheumatologists. As this system is not applied world-wide, there are large global differences in availability and accessibility of rheumatologic care. The CARE tool may be used to overcome these differences, but studies in other countries are warranted to evaluate the performance of the CARE tool in other regions.

Finally, the simulation that was performed in c**hapter 2** used an estimation of the prevalence of IA when GPs were in doubt to mimic a primary care setting. This estimation was derived from all available literature on this subject (and was set at 20%), but was based on only two studies.[24,25] Future studies will need to investigate the actual prevalence of IA when the GP feels uncertain about its presence.

Another avenue of research to potentially extend knowledge in the field of rheumatology could be a study of cost-effectiveness of early recognition. If the 'window of opportunity' hypothesis is correct, then there is a confined period in which RA is most susceptible to the disease-modifying effects of treatment. Consequently, one would expect that patients that have been recognized within this window need less second- or third-line treatment options. Since second- or third line options often include expensive biological DMARDs (e.g. adalimumab, etanercept) or targeted synthetic DMARDs (e.g. tofacitinib or baricitinib), a big economic benefit could possibly be made. However, increased early recognition will require more time-investment of rheumatologists and therefore the exact yield is still to be determined.

The main limitation of **chapter 3** was the number of patients that could be studied in the stratified subgroups according to autoantibody combinations. Studying only a limited number of patients will lead to small statistical power and thus introduces the possibility of erroneously failing to reject a null hypothesis that is actually false (type II error). Despite a larger number of patients and a longer duration of follow up than a previous study investigating the risk conferred by ACPA in CSA patients[21], more accurate hazard ratios and absolute risks could potentially be calculated by inclusion of larger numbers.

Future studies investigating accurate identification of patients at risk for RA will likely look into new biomarkers of disease. For this end, further understanding of the pathophysiology of RA is needed. In recent years, another post-translational modification of proteins[5], carbamylation, was identified to be affected in the early phases of RA. The role of this novel autoantibodies (anti-carbamylated protein antibody) in the diagnostic workup of RA will need to be investigated in future studies to assess if it will become the next autoantibody to regularly test in addition to RF and ACPA, as is now common practice in Western countries. The letter in **chapter 4** showed that testing anti-CarP as a third autoantibody in

patients with CSA had limited added value for positive predictive value of development of clinical arthritis. Future studies in disease stages that precede CSA could investigate if testing anti-CarP as a third autoantibody has additional value to RF and ACPA, for instance by screening individuals to identify those at high(est) risk for developing RA.

Furthermore, the value of these autoantibodies can be different in the different stages of RA. Future population-based cohort studies will have to demonstrate if a combination of three autoantibodies can contribute to the identification (screening) of at-risk patients before the onset of arthralgia.

With structural joint damage becoming increasingly rare, the position of an (if not the most) important outcome measure has shifted towards functional ability of patients. As demonstrated in **chapter 5**, CSA patients already suffer from increased functional disability as compared to the general population. This demonstrated the impact of RA on the daily living of RA patients, even in its earliest phases. Functional disability did not further increase after identification of clinical arthritis, demonstrating the relevance of CSA from patients' perspectives. This is an interesting opposition of the clinician's perspective, where the identification of clinical arthritis is the key and forms the start sign to initiate treatment. To study if this opposition is also present for other patient-reported outcomes (fatigue, work ability, health-related quality of life, etc.), future studies in CSA patients could be performed of these outcome measures.

The most important outcome measures in future studies of RA remain to be determined. One might wonder if these need to encompass objective outcome measures, such as abnormalities in laboratory findings or imaging abnormalities. Otherwise, more subjective patient-reported measures could be considered, which comprise pain, health-related quality of life or stress. Future studies could look into developing entirely new instruments of assessing outcomes. A distinction between "generic" (suitable for

many diseases and conditions) and "disease-specific" (limited to use in one or a few disease conditions) instruments needs to be appraised when developing a new tool/questionnaire to assess patient outcomes.

Despite increasing rarity, structural joint damage still is an important outcome measures in RA research. Structural damage is caused by a discrepancy between bone formation and bone loss, and articular bone erosions (as evaluated by MRI) were evaluated in **chapter 6**. Future longitudinal studies could look into the value of erosions as detected by MRI in CSA patients. For instance, it could be investigated if an erosion as observed on MRI can be predictive for future erosions or joint destruction that can be observed on radiographs in patients that develop RA. However, it needs to be noted that a large number of patients will need to be studied over time for this end as erosions are increasingly rare.

Furthermore, considering that **chapter 6** was an association study, the biological mechanism could not be investigated. Elucidating this mechanism may be a subject of new studies unraveling the mechanism of articular joint erosions.

All the longitudinal studies investigating MRI-detected subclinical inflammation in this thesis (**chapter 7–9**) have the limitation of small absolute patients numbers. For conclusions with more statistical power, larger groups (especially the number of ACPA-positive RA patients) will be needed. Larger patient numbers could enable more detailed sensitivity analyses that compare autoantibody-positive patients with antibody-negative patients, which could enhance insight in disease processes in the earliest phases of RA.

Interestingly, as was established in **chapter 9**, not all patients with CSA will progress to RA and spontaneous resolution of CSA was possible in about

27% of CSA patients over time. This is of interest to further understand the mechanisms underlying development of RA. Future studies could look into the (biological) mechanisms that work in patients that despite having subclinical inflammation as depicted by MRI will not convert to clinical arthritis.

Ideally, validation should be performed of the results from all the longitudinal MRI-studies investigating disease mechanisms in this thesis. If MRIs could be performed at more frequent time points, the course of MRI-detected subclinical inflammation in the phases preceding identification of RA could be studied in more detail.

With an increasing number of tests and biomarkers available, the physical and physiological burden these tests could place on a patient must not be forgotten.[26] Future qualitative and quantitative studies may look into disease perceptions and cognitions of patients in the preclinical phases.

Nonetheless, with early identification likely further improving over the coming decennia, the research agenda will likely shift to treatment in increasingly early phases of RA. The 'window of opportunity' hypothesis presumes the existence of an early period in which RA is most susceptible to DMARD treatment. Right now, DMARD-free sustained remission functions as the best proxy available to actually curing RA. Future research will show if treatment in these early phases will lead to delay of the occurrence of RA, or that disease processes will be altered in such a way that manifestation of RA can be prevented in its entirety. Several trials are currently ongoing in the world that will look into starting RA treatment in these early phases.[27–31] The results are expected to arrive within 5 years and will likely determine the guidelines of treatment for decades to come.

For subsequent studies, a shift towards using real-world data from patient files could be strived for. Patient data is currently stored in electronic files, which poses a (potential) goldmine of data yet to be delved. Nonetheless, cohort studies and clinical trials will remain instrumental in the future of research, as these studies can specify clear inclusion and exclusion criteria to select and analyse harmonized groups of patients. However, if world- or continent-wide initiatives can be launched to share mined anonymized data from patients files, the number of patients that can be studied could in theory be very large. The consequent statistical power that can be generated could allow observations in groups of patients that normally couldn't be studied due to their rarity. The generalizability of real-life data is an important deliberation, but reduction of "noisy data" (by filtering procedures or standardized registration) will be a challenge. Physicians will in the future need to be aware that whatever is entered in patient files may at a later point anonymous be mined for research purposes. However, before mining of data from patient files is possible, strict regulations will need to be implemented to ensure the patients' privacy.

Overall, most importantly, there remains a need to replicate the results of this thesis. Possibly, other centres in the Netherlands – or elsewhere in the world – could establish similar but independent cohorts of CSA patients to this end.

In conclusion, this thesis showed that although early identification is increasingly improving, there remains a large proportion of patients that cannot be accurately identified despite a suspect pattern of signs and symptoms, as well as information on autoantibodies. Furthermore, the burden of disease is already substantial during the symptomatic pre-arthritis phase of CSA. Future studies will have to provide evidence for the effectiveness of preventing persistent RA and functional disability with prescribing DMARD treatment in the phase of CSA.

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Chapter 11

Nederlandse samenvatting

Introductie

Reumatoïde artritis

Reumatoïde artritis (RA) is een chronische, inflammatoire gewrichtsziekte die gekenmerkt wordt door persisterende synovitis en systemische inflammatie.[1] Er kan permanente schade ontstaan aan de gewrichten door destructie van kraakbeen en bot.[1] Indien onbehandeld, dan zal RA in de meerderheid leiden tot verslechtering van het fysiek functioneren, het kunnen uitvoeren van alledaagse taken en aanleiding geven tot een verminderde arbeidsparticipatie en -productiviteit.

RA is één van de meest frequent voorkomende inflammatoire gewrichtsziekten, en geschat wordt dat ongeveer 1% van de bevolking door RA aangedaan is.[2] De typische presentatie van "klassieke" RA is een vrouw van middelbare leeftijd, met subacute sluimerende polyarticulaire en symmetrische artralgie en zwelling van de kleine hand- en voetgewrichten. Doorgaans zijn de metacarpophalangeale (MCP), proximale interphalangeale (PIP), pols en metatarsophalengeale (MTP) gewrichten aangedaan. Andere kenmerken klachten en symptomen omvatten ochtendstijfheid, moeheid en gewichtsverlies. Verder kunnen ook extra-articulaire manifestaties bestaan, zoals huidafwijkingen (reumanoduli), pulmonale of cardiale betrokkenheid, verminderd mentaal welbevinden en vasculitis.[3,4] Bij het lichamelijk onderzoek moet altijd gekeken worden naar de aanwezigheid en verdeling van pijnlijke en gezwollen gewichten. Het laboratoriumonderzoek kan aanwijzing geven voor verhoogde concentraties van autoantistoffen: anti-citrullinated protein antibodies (ACPA of anti-CCP) en reumafactor (RF). Verder kunnen ook acute fase eiwitten zoals C-reactief protein (CRP) en de bezinkingssnelheid van erytrocyten (BSE) verhoogd zijn in RA.

Vroege identificatie van RA

Het vroegtijdig herkennen van RA is een belangrijk speerpunt binnen de

reumatologie, omdat dit de mogelijkheid zal bieden tot het tijdig starten van behandeling. De update van de EULAR aanbevelingen uit 2016 voor behandeling van RA geeft aan dat disease-modifying antirheumatic drugs (DMARDs) direct gestart moeten worden zodra de diagnose RA gesteld is.[5] De onderliggende gedachte hieraan is de mogelijkheid van een "window of opportunity"; een vroege ziekteperiode waarin RA het meest gevoelig is voor het effect van behandeling. De huidige consensus is dat DMARDS het beste effect hebben als de onderliggende ziekteprocessen nog niet volledig uitgerijpt zijn. Omgekeerd is aangetoond dat het later starten van behandeling geassocieerd is met slechtere ziekte-uitkomsten, waarbij er meer schade aan gewrichten werd gezien en lagere percentages van remissie van ziekte werden bereikt.

Echter, het vroeg herkennen van RA is een uitdaging voor clinici in zowel eerste als tweede lijn. Met name in de eerste lijn is er een hoge incidentie van consultatie voor gewrichtsklachten, terwijl de incidentie van daadwerkelijk RA juist laag is.[6] Hoewel de prevalentie van consultatie voor gewrichtsklachten ongeveer 2405 per 10,000 per jaar bedraagt[6,7], zal de gemiddelde fulltime huisarts slechts één nieuwe patiënt met RA zien per jaar.[8]

Om de uitdaging van correcte vroegherkenning van RA te ondervangen zijn in de literatuur in recente jaren verschillende strategieën voorgesteld voor het identificeren van "at risk" groepen. Zo omvat één strategie de identificatie van deze "at risk" groep door middel van het testen van aanwezigheid van autoantistoffen (ACPA en RF) in het serum. Als deze autoantistoffen aanwezig zijn wordt de betreffende persoon als "at risk" beschouwd voor het ontwikkelen van RA.[9,10] Een voordeel van deze strategie is dat het makkelijk toe te passen is, ook in de eerste lijn, omdat evaluatie door een reumatoloog niet noodzakelijk is voorafgaand aan het inzetten van labonderzoek. Een nadeel is dat een deel van de patiënten die RA zullen ontwikkelen deze autoantistoffen (nog) niet hebben, en daarmee onterecht als niet "at risk" zullen worden beschouwd.

Een andere strategie is het identificeren van patiënten "at risk" door gebruik te maken van klinische evaluatie door een reumatoloog. Deze zal dan op basis van klinische expertise en door middel van patroonherkenning in anamnese en lichamelijk onderzoek een patiënt inschatten als zijde "at risk". De herkenning van een zeker klachtenpatroon zal – al dan niet onbewust – door reumatologen in de dagelijkse kliniek reeds toegepast worden en kan leiden tot de identificatie van patiënten met Clinically Suspect Arthralgia (CSA). Slecht een klein deel van alle patiënten die door een reumatoloog in de tweede lijn geëvalueerd worden voldoen aan dit specifieke klachtenpatroon: <6%.[11] Pas na herkenning van CSA zullen laboratoriumonderzoek en verder aanvullend onderzoek toegepast worden. Een voordeel van deze aanpak is dat zowel patiënten met als zonder autoantistoffen bestudeerd kunnen worden, alsook de mogelijkheid tot het onderzoek van het onderscheidend vermogen van aanvullende diagnostiek door middel van bloedonderzoek of beeldvorming. Een nadeel van deze aanpak is dat het een zekere mate van subjectiviteit omvat omdat CSA omkaderd is door de klinische expertise van de betreffende reumatoloog.

Om inclusie van homogene groepen patiënten tussen verschillende studies te kunnen waarborgen heeft een EULAR taskforce recent een definitie opgesteld voor artralgie die verdacht is voor progressie tot RA.[12] De betreffende karakteristieken staan benoemd in Tabel 1.

Tabel 1. EULAR definitie van karakteristieken van artralgie "at risk" voor het ontwikkelen van RA

Anamnese:

- Kort bestaande gewichtsklachten (duur <1 jaar)
- Symptomen gelokaliseerd in MCP gewrichten
- Duur van ochtendstijfheid ≥60 min
- Symptomen het ergst in de vroege ochtend
- Positieve eerstegraads familieanamnese voor RA

Lichamelijk onderzoek:

- Moeite met het maken van een vuist
- Positieve "squeeze test" van de MCP gewrichten

Het CSA cohort

In dit proefschrift is veelvoudig gebruik gemaakt van data verkregen uit het CSA cohort van het Leids Universitair Medisch Centrum (Leiden, Nederland). In dit inceptiecohort zijn sinds 2012 patiënten geïncludeerd die op basis van hun klachtenpatroon door hun reumatoloog verdacht werden van RA in wording. Per definitie hadden alle patiënten kort bestaande (<1 jaar) artralgie aan de hand- (MCP, PIP of pols) of voetgewrichten (MTP) en werden zij beschouwd als "at risk" voor het ontwikkelen van RA op basis van hun eerste bezoek aan de reumatoloog. Omdat CSA beschouwd wordt als het ziektestadium vóór het daadwerkelijk ontstaan van de kenmerkende gewrichtsontsteking (artritis) van RA, hadden patiënten géén CSA als artritis reeds aanwezig bij het eerste bezoek. Dientengevolge zouden patiënten met reeds bestaande artritis niet geïncludeerd worden in het CSA cohort. Een andere reden om niet geïncludeerd te worden in het CSA cohort was als er een duidelijk andere verklaring bestond voor de gewrichtsklachten, waarbij te denken valt aan tekenen van fibromyalgie (aanwezigheid van tenderpoints) of artrose (bijvoorbeeld Heberden of Bouchard noduli).

Aangezien huisartsen in de regio van het LUMC ontraden werd om te testen op de aanwezigheid van autoantistoffen vóór verwijzing naar een reumatoloog waren de ACPA- en RF-status vaak onbekend bij eerste

presentatie. Bij het eerste bezoek werd, na het verkrijgen van informed consent en inclusie in het CSA cohort, verder aanvullend onderzoek ingezet door middel van laboratoriumonderzoek en beeldvorming (röntgenfoto's en MRI).

Patiënten werden daarna prospectief gevolgd met geplande bezoek op 4, 12 en 24 maanden na het eerste bezoek. Indien nodig (bijvoorbeeld als een patiënt verergering van de klachten ervaarde of een gezwollen gewricht bemerkte) werden zij tussendoor gezien door de reumatoloog. Zodoende werd gewaarborgd dat het ontstaan van artritis bij de eerste mogelijkheid werd herkend. Follow-up in het CSA cohort werd beëindigd als artritis werd vastgesteld, of anders na het laatste bezoek op 24 maanden.

De patiënten in het CSA cohort werden geïncludeerd op basis van een klinische verdenking door de reumatoloog. Het voldoen aan de bovengenoemde EULAR definitie voor artralgie "at risk" voor RA[12] was dus geen voorwaarde, maar de betreffende kenmerken werden wel bekeken en konden dus achteraf toegepast worden.

Doelen van dit proefschrift

Dit proefschrift heeft zich toegelegd op de vroege fases van Reumatoïde Artritis (RA). Omdat voorgaande studies lieten zien dat individuen met positieve autoantistoffen in ongeveer 30–50% daadwerkelijk converteerden tot RA[9,13–16], bleef er een noodzaak bestaan tot het meer accuraat kunnen identificeren van RA in wording. Verder is gekeken naar de impact die de vroege ziektefase van CSA heeft op de functionele beperkingen die ervaren worden door patiënten. Tot slot hebben we onderzoek gedaan naar de verschillende processen die van invloed zijn in de vroege fases van RA.

Verder is in dit proefschrift gekeken naar de ziektelast, en is getracht het begrip van de mechanismen in de vroegste fasen van RA beter te begrijpen. Veel van de studies in dit proefschrift hebben zich gefocust op data afkomstig uit het CSA cohort in het Leids Universitair Medisch Centrum.

Deel 1: Progressie tot RA en geassocieerde ziektelast

In het eerste deel van dit proefschrift, te weten **hoofdstukken 2, 3 en 4**, is de focus gelegd op de factoren die bijdragend kunnen zijn in de identificatie van RA en progressie tot RA in haar vroegste fasen. **Hoofdstuk 5** evalueerde de ziektelast gedurende progressie tot RA.

Ondanks het onderkennen van de aanbevelingen in nationale en internationale richtlijnen tot het snel verwijzen van patiënten met mogelijke inflammatoire artritis (IA), geven huisartsen nog steeds aan zich niet zeker te voelen in hun eigen vaardigheden tot het opsporen van synovitis door middel van gewichtsonderzoek.[17] Gezien de noodzaak tot vroege identificatie van RA, betrof het doel van **hoofdstuk 2** het ontwikkelen en valideren van een klinische beslisregel, bestaande uit klinische karakteristieken, waarbij de huisarts geholpen kan worden met het herkennen van IA wanneer hij/zij in twijfel is.

Dit onderzoek werd verricht door middel van data verkregen uit de Leidse Early Arthritis *Recognition* Clinic (EA*R*C); een screeningskliniek waarbij patiënten verwezen kunnen worden wanneer de huisarts IA vermoedde. In dit dwarsdoorsnedeonderzoek werden associaties gevonden met het ontwikkelen van IA voor de volgende variabelen: het mannelijk geslacht, een leeftijd ≥60 jaar, een symptoomduur van <6 weken, ochtendstijfheid >60 minuten, een laag aantal pijnlijke gewrichten (1–3 gewrichten), de aanwezigheid van patiënt-gerapporteerde gewrichtszwelling en moeite met het maken van een vuist. Voor gebruik in de dagelijkse kliniek van de huisarts werd een model gemaakt waarbij de verkregen β's uit het multivariabele regressiemodel werden versimpeld door af te ronden naar de dichtstbijzijnde 0.5. Dit resulteerde in een beslisregel bestaande uit 7 items en een score lopend van 0 tot 7.5 met bijbehorende voorspelde risico's op IA. De beslisregel had een redelijk onderscheidend vermogen voor IA en zou huisartsen kunnen bijstaan bij het maken van beslissingen bij patiënten

waarbij IA wordt vermoed, zodat de juiste zorg ingezet kan worden.

Het onderzoek bevat ook een simulatie van de beslisregel waarin de prevalentie van inflammatoire artritis op 20% werd gezet. Deze simulatie werd verricht om de toepasbaarheid van de regel na te bootsen in een eerstelijnssetting, aangezien er in deze setting waarschijnlijk een lagere prevalentie zal zijn dan in de EA*R*C (gesitueerd tussen de eerste en tweede lijn). Onze beslisregel is beschikbaar gemaakt in een webapplicatie waarbij voorspelde risico's op het bestaan van IA worden gegeven. De zogenoemde CARE tool, zoals gepresenteerd in **hoofdstuk 2**, onderscheid zich van andere studies waarin regels werden ontwikkeld in het feit dat zij zich niet richt op het triëren van patiënten die reeds naar de tweede of derde lijn zich verwezen.[18–20] De CARE tool richt zich daarentegen juist op het assisteren in het maken van beslissingen en het eventueel inzetten van een verwijzing voor de juiste patiënten. De beslisregel richt zich op het faciliteren van vroege herkenning van IA (aangezien hier snelle verwijzing naar een reumatoloog nodig zal zijn), en is dus géén longitudinale studie waarbij voorspelling van een specifieke diagnose (bijvoorbeeld RA) op een later punt wordt beoogd.

In **hoofdstuk 3** werden risico's op het ontwikkelen van klinische artritis onderzocht voor drie verschillende autoantistoffen: ACPA, RF en/of anti-carbamylated protein antibodies (anti-CarP). ACPA en RF worden in de reguliere zorg getest bij (vrijwel) alle patiënten met RA. Anti-CarP is een nieuwe autoantistof waar veel onderzoek naar is verricht in recente jaren.[21] Wanneer onafhankelijk van elkaar beschouwd werden associaties gevonden met het ontwikkelen van klinische artritis voor zowel ACPA, RF als Anti-CarP. Echter, wanneer er gecorrigeerd werd voor het gelijktijdig aanwezig zijn van deze drie antistoffen was alleen ACPA nog significant geassocieerd met het ontwikkelen van artritis. De positief voorspellende waarden (in het Engels: "positive predictive value" of "PPV") voor het ontwikkelen van artritis binnen 2 jaar waren: 63% voor ACPA, 53% voor RF en 50% voor anti-CarP. Het gelijktijdig aanwezig zijn van ACPA en RF

in een patiënt gaf een PPV van 67%. Hoewel hogere titers van ACPA niet geassocieerd waren met hogere kansen op het ontwikkelen van artritis, gold dit wel voor RF. Onze data impliceren dat ACPA het hoogste risico met zich meebrengt voor het ontwikkelen van RA, en bovendien van toegevoegde waarde is bovenop het testen van RF. Desondanks moeten zowel clinici als onderzoekers zich bewust zijn van het omgekeerde van de PPV van 67% van ACPA en RF. Dit zal betekenen van nog altijd >30% van de artralgie patiënten die zowel ACPA- als RF-positief zijn geen RA zullen ontwikkelen. Dit deed ons concluderen dat het aanwezig zijn van CSA en antistoffen nog altijd onvoldoende accuraat is om antistof-positieve RA te voorspellen.

Het dient opgemerkt te worden dat PPVs afhankelijk zijn van de prevalenties van "cases" in cohortstudies, wat wil zeggen dat dezelfde (serum)test andere resultaten kan geven afhankelijk van de setting waarin deze getest wordt. Patiënten die geschouwd worden als CSA door hun reumatologen zijn slechts een kleine groep van alle patiënten die zich met artralgie presenteren in de tweede lijn (<6%).[11] Dientengevolge hebben de onderzochte patiënten uit **hoofdstuk 3** een hogere kans op het ontwikkelen van RA dan patiënten met non-specifiek artralgie in de eerste of tweede lijn. In **hoofdstuk 4** zijn de implicaties onderzocht van het testen van twee of drie autoantistoffen. Daarbij werd geen toegevoegde waarde gevonden van het testen van een aanvullende autoantistof in toevoeging tot ACPA en RA in patiënten met CSA.

In **hoofdstuk 5** zijn de functionele beperkingen onderzocht die gerapporteerd werden door patiënten met CSA. Hierbij hebben we gevonden dat de scores van functionele beperking, onderzocht middels de Health Assessment Questionnaire Disability Index (HAQ-DI) al verhoogd waren in de fase van CSA: 0.50 voor alle patiënten. Deze score kwam overeen met de HAQ-DI gerapporteerd in een andere studie met ACPA-positieve artralgie patiënten.[22] Een score van 0 op de HAQ-DI is een indicatie van geen moeilijkheden met het uitvoeren van functionele

taken, waar 1 een indicatie is van enige moeilijkheid. Bovendien vonden we dat hogere scores van inflammatie (zoals gedetecteerd met MRI) geassocieerd waren met meer functionele beperkingen. Interessant genoeg vonden we dat, gedurende follow-up, dat verhoogde HAQ-DI scores (≥1.0) geassocieerd waren met het later ontwikkelen van klinische artritis. Eerdere studies in patiënten met vroege RA lieten zien dat de gemiddelde HAQ-scores hier ook ongeveer 1.0 waren.[23,24] Een eveneens interessante bevinding was dat scores van functionele beperkingen niet verder toename van het moment van presentatie met CSA tot het daadwerkelijk ontwikkelen van artritis (0.88 en 0.75, respectievelijk). Samengenomen laten deze data zien dat functionele beperking al van eenzelfde ernst waren in de symptomatische pre-artritis fase van CSA als de vroegste fasen van RA, waarbij het maximum aan beperkingen in deze fasen als bereikt was bij eerste presentatie met CSA. Deze bevindingen kunnen als illustratie dienen van de relevantie van de artralgie fase vanuit het perspectief van de patiënt.

Als conclusie van dit deel van het proefschrift geldt dat betere vroegherkenning van RA (mogelijkerwijs) bereikt kan worden door het gebruik van de CARE beslisregel, mogelijk verreikt door kennis over andere klinische kenmerken of wellicht autoantistoffen. Desondanks kan een aanzienlijk deel van de patiënten met antistof-positieve ziekte in wording nog onvoldoende herkend worden ondanks de aanwezigheid van een suggestief patroon van klinische tekenen en symptomen en de aanwezigheid van autoantistoffen en functionele beperkingen.

Deel 2: Ziekteprocessen in de ontwikkeling van RA

In het tweede deel van het proefschrift hebben we getracht meer inzicht te verkrijgen in de ziekteprocessen in de vroege fasen van RA door onderzoek te doen naar de biologische mechanismen (**hoofdstuk 6**), en hebben we longitudinale studies gedaan door middel van MRI en inflammatie bekeken op zowel het gewrichts- (**hoofdstuk 7**) als het patiënt-niveau (**hoofdstuk 8**) over tijd.

Van ACPAs wordt gedacht dat zij directe activatie geven van osteoclasten, wat zal leiden tot erosies (en pijn) in RA patiënten. In **hoofdstuk 6** zochten we bewijs voor deze theorie in mensen, waar eerdere studies vooral in proefdieren verricht waren. Het hoofdstuk evalueerde of, in patiënten met artralgie, ACPA geassocieerd was met erosies, al dan niet onafhankelijk van onderliggende inflammatie of het gelijktijdig aanwezig van RF. Zoals verwacht hadden ACPA-positieve patiënten hogere scores voor erosies op MRI dan ACPA-negatieve patiënten. Echter, dit proces lijkt gemedieerd te worden door gelijktijdige aanwezigheid van inflammatie: ACPA-positieve patiënten zonder inflammatie hadden een gelijke erosie-score als ACPA-negatieve patiënten. Dit in tegenstelling tot ACPA-positieve patiënten met lokale inflammatie, waarin significant hogere erosie scores werden geobserveerd. Een mediatie analyse toonde aanwijzingen dat inflammatie in het causale pad ligt van ACPA leidend tot hogere erosie scores. In een analyse met drievoudige stratificatie werd aangetoond dat ACPA en/of RF alleen met hogere inflammatie-scores geassocieerd zijn indien gelijktijdige inflammatie aanwezig is.

Van de patiënten die IA zullen ontwikkelen is onbekend hoe het verloop van inflammatie op gewrichtsniveau zich afspeelt. In **hoofdstuk 7** werd MRI-gedetecteerde subklinische inflammatie bekeken over tijd. Er werden een tweetal theorieën onderzocht: of artritis een lokaal uitgroeiend proces is, of dat er meer gedacht dient te worden aan een systemisch probleem

waarbij locaties van lokale subklinische inflammatie grotendeels los staat van de gewrichten waarin artritis zal ontstaan.

In deze studie werden 350 gewrichten onderzocht van 35 patiënten met CSA (die allen zouden converteren tot artritis in ten minste één gewricht). Van alle gewrichten waarin uiteindelijk synovitis zou worden vastgesteld, was in 69% van de gevallen geen subklinische inflammatie te zien tijdens het baseline bezoek. Dit zou het beste passen bij de theorie van een systemisch proces, wat grotendeels ondersteund wordt door voorgaande studies waarin reeds verhoogde markers van systemische inflammatie worden gevonden in het laboratoriumonderzoek in de pre-artritis fase.[25]

Net als in hoofdstuk 7 werd in **hoofdstuk 8** gekeken naar het verloop van MRI-gedetecteerde subklinische inflammatie tijdens progressie tot RA, alleen in dit geval op het patiëntniveau. De twee geldende theorieën over ontwikkelen van artritis zijn de "outside-in hypothese" [26–29] (waarbij synovitis voorafgaat aan betrokkenheid van de botten) en de "inside-out hypothese" [27,30,31] (waarbij RA gezien wordt als primaire ziekte van het beenmerg en de synoviale membraan pas later in de ziekte aangedaan zal zijn). Om deze uitstaande vraag te onderzoeken werd gekeken naar patiënten die allen converteerden van CSA naar RA. Bij presentatie met CSA waren met name de synovitis- en tenosynovitis-scores hoger in CSA patiënten dan in gezonde controles. Tijdens de progressie tot RA werden met name toename in de scores gezien van synovitis en beenmerg oedeem. Dit patroon, waarbij synovitis en tenosynovitis eerst verhoogd waren, en pas later beenmerg oedeem zichtbaar werd past het beste bij de "outside-in theorie" in de ontwikkeling van RA. Het behoeft nog wel verder onderzoek of dit geldt voor zowel ACPA-positieve als ACPA-negatieve patiënten.

Tot slot is er nog veel onbekend over de uitkomsten in patiënten die niet zullen converteren tot RA. In **hoofdstuk 9** werd het ziektebeloop onderzocht in deze patiënten, alsook de samenhang tussen het beloop

van symptomen en MRI-gedetecteerde subklinische inflammatie. Na twee jaar follow-up had een derde van de patiënten resolutie van symptomen, terwijl twee derde juist persisterende symptomen ervaarde. Opvallend was dat de baseline totale MRI-inflammatie scores in de patiënten die resolutie ervaarden significant hoger waren dan scores in gezonden controles, terwijl dit niet gold voor de patiënten zonder resolutie. Na twee jaar was in beide groepen geen verschil meer te zien met gezonde controles, waarbij de scores van patiënten met resolutie van symptomen ook een significante daling toonden over de tijd. Samengenomen lieten onze data zien dat resolutie van symptomen mogelijk is na een fase van CSA en dat de patiënten die resolutie van hun symptomen ervaarden ook resolutie van subklinische inflammatie hadden.

Conclusies en toekomstperspectieven

In recente decennia zijn er grote stappen geboekt in de vroege identificatie en behandeling van patiënten met RA. Door deze vooruitgangen zijn de uitkomsten op lange termijn van deze patiënten drastisch verbeterd. Waar een veertigtal jaar geleden de behandeling van RA nog bestond uit rust, fysiotherapie en analgetica, zijn de rolstoelen en ernstige gewrichtsdeformaties bijna verdwenen uit de wachtkamers bij de Reumatologie. Vroegherkenning van ziekte heeft hier een grote bijdrage aan geleverd en de afgelopen jaren heeft het Clinically Suspect Arthralgia (CSA) cohort hier een belangrijk onderdeel van uitgemaakt. Voor de herkenning van een patiënt met CSA is klinische expertise benodigd, waarbij een patroon van gewrichtsklachten, functionele beperkingen en ochtendstijfheid gedestilleerd kan worden. Ook het lichamelijk onderzoek maakt een belangrijk deel van het reumatologisch onderzoek uit, waarbij het gewrichtsonderzoek nog altijd als gouden standaard geldt voor het vaststellen van synovitis.

Juist het herkennen van het klachtenpatroon en het gewrichtsonderzoek is iets wat door huisartsen zelf als lacune in de eigen expertise wordt aangegeven. Validatie van de resultaten uit hoofdstuk 2 in de eerstelijns setting is daarom van belang. Zoals ook in hoofdstuk 2 reeds aangegeven zou de discriminatoire waarde van de voorspelregel nog verder verbeterd kunnen worden door het toevoegen van extra variabelen. Hierbij valt te denken aan de “squeeze test” van MCP gewrichten, of afwijkingen op beeldvormend onderzoek. Ook dit zou verder onderzocht kunnen worden in een echte setting van de eerste lijn.

Het systeem waarin de Nederlandse huisartsen functioneren als poortwachter voor de tweede lijn wordt niet wereldwijd toegepast, en dientengevolge zijn er grote verschillen aan te wijzen in de beschikbaarheid en toegankelijkheid van reumatologische zorg. Hoewel de CARE tool uit

hoofdstuk 2 zou kunnen functioneren als verlaging van de drempel voor beschikbaarheid van zorg in de tweede lijn, moet de validiteit van de regel verder onderzocht worden in andere regio's wereldwijd.

Tot slot is in hoofdstuk 2 een simulatie verricht waarbij een schatting werd gemaakt op de prevalentie van IA wanneer huisartsen twijfelden. Deze schatting is afgeleid uit eerdere literatuur, maar baseert zich slechts op 2 studies.[32,33] Toekomstige studies zouden verder kunnen onderzoeken hoe vaak IA daadwerkelijk aanwezig is in het geval van twijfel bij de huisarts.

Een andere onderzoeksrichting voor de toekomst van de reumatologie zou zich kunnen richten op de kosteneffectiviteit van de vroege herkenning van RA. Als de hypothese over een "window of opportunity" klopt, dan zou verwacht mogen worden dat patiënten die juist binnen dit tijdsbestek correct herkend worden als RA minder vaak tweede of derdelijns behandeling nodig zullen hebben, zoals biological DMARDs of de nieuwe targeted synthetic DMARDs. Echter, extra inspanning in vroegherkenning zal ook meer tijd vereisen van de reumatoloog, waarbij de precieze netto opbrengst nog onderzocht moet worden.

De belangrijkste limitatie van een aantal analyses in dit proefschrift was het beperkte aantal patiënten dat onderzocht kon worden, met name in de gestratificeerde subgroepen. Bij onderzoek met kleine patiëntengroepen bestaat er – door kleine statistische "power" – een grotere kans op het onterecht verwerpen van de nulhypothese (type II fout). Inclusie van grotere groepen patiënten in de toekomst zou tot een grotere power moeten leiden, met als gevolg meer accurate hazard ratio's en absolute risico's.

De belangrijkste uitkomstmaten in toekomstige studies over RA staat nog ter discussie. Enerzijds zouden de uitkomstmaten vooral objectieve

maten moeten omvatten, zoals afwijkingen in het labonderzoek of op beeldvorming. Anderzijds zouden ook subjectieve (door de patiënte gerapporteerde) maten bekeken moeten worden; te denken valt aan pijn, kwaliteit van leven, ervaren stress of functionele beperkingen. Ook hier zou verder onderzoek naar verricht kunnen worden.

Voorts bevatte dit proefschrift meerdere onderzoeken waarbij vooral wordt gekeken naar associaties op het niveau van patiënten en groepen van patiënten. Hierbij valt te denken aan, bijvoorbeeld, de associatie tussen inflammatie en erosies in hoofdstuk 6. Deze studies lieten niet toe om het onderliggende biologische mechanisme verder te onderzoeken. Toekomstige studies zouden zich hier op kunnen richten.

Verder zouden er onderzoeken verricht kunnen worden ter validatie van de verschillende longitudinale studies die betrekking hadden op inflammatie te zien is met MRI. Zo zouden deze studies ook MRI-scans kunnen verrichten op meer frequente tijdspunten om de relaties over tijd in meer detail te kunnen bekijken.

Verder dient niet te vergeten te worden dat het steeds groter wordende arsenaal aan testen en biomarkers ook een grotere druk op de patiënt legt. [34] Zowel kwalitatief als kwantitatief onderzoek zou verricht kunnen worden om meer te weten te komen over ziekteperceptie en cognitie van patiënten in de preklinische fasen van RA.

Niettemin zal, door de steeds beter wordende vroege herkenning van RA, de onderzoeksagenda zich op gaan schuiven naar behandeling in steeds vroegere fasen van RA. Op dit moment is aanhoudende remissie van ziekte zonder de noodzaak tot het gebruiken van DMARDs (“DMARD-free sustained remission”) de beste afgeleide beschikbaar voor het daadwerkelijk genezen van RA. Door vroeger te behandelen hopen

onderzoekers in de komende jaren, in verschillende trials[35–39], bewijs te verzamelen dat het optreden van RA in het geheel voorkomen kan worden. Resultaten van deze trials zullen in de komende 5 jaar gaan verschijnen en zullen waarschijnlijk bepalend gaan zijn voor de richtlijnen voor de behandeling van RA van de komende decennia.

Tot slot blijft voor alle onderzoeken in dit proefschrift gelden dat validatie van resultaten, zoals dat voor de gehele wetenschap geldt, noodzakelijk is.

In conclusie is in dit proefschrift aangetoond dat hoewel de vroege identificatie van RA steeds verder verbeterd wordt, er nog altijd een grote groep patiënten bestaat die niet accuraat herkend kan worden ondanks een kenmerkend patroon van klachten, en de beschikbaarheid van informatie over aan- of afwezigheid van antistoffen. Verder is de ziektelast van RA al verhoogd in de pre-artritis fase van CSA. Toekomstige studies zullen het bewijs moeten gaan leveren wat de mogelijkheden zijn tot het voorkomen van persisterende RA en functionele klachten door het voorschrijven van behandeling met DMARDs tijdens de fase van CSA.

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Appendices

Curriculum Vitae

Robin Marco ten Brinck werd op 13 oktober 1989 geboren als eerste kind van Marcel ten Brinck en Karin ten Brinck-de Winter. Hij groeide op in Den Haag. In 2008 deed hij eindexamen VWO op het Hofstad Lyceum in Den Haag. Direct aansluitend begon hij aan de studie Luchtvaart- en Ruimtevaarttechniek aan de Technische Universiteit Delft. Na één jaar maakte hij in 2009 de overstap naar het Leids Universitair Medisch Centrum (LUMC) waar hij begon aan zijn studie Geneeskunde. Naast zijn studie bracht hij veel tijd (al dan niet roeiend) door op de A.L.S.R.V. Asopos de Vliet. Daar leerde hij ook Mirjam Zeilmaker kennen. In 2014 leidde een extracurriculair onderzoeksproject bij de afdeling Urologie tot twee onderzoeksprijzen voor "Best Student Researcher". Aan het eind van de coschappen deed hij een semi-arts stage bij de afdeling Reumatologie van het LUMC. In oktober 2015 rondde hij de studie Geneeskunde af en begon hij aan een promotietraject bij de afdeling Reumatologie van het LUMC, waar hij onderzoek deed naar vroegherkenning van reumatoïde artritis onder begeleiding van prof. dr. A.H.M. van der Helm-van Mil en prof. dr. T.W.J. Huizinga. Na zijn promotietraject is hij begonnen als arts-assistent bij de afdeling Interne Geneeskunde van het Groene Hart Ziekenhuis in Gouda, alwaar hij de vooropleiding van het traject van de opleiding tot Reumatoloog is begonnen.

List of publications

1. de Jong Y, Pinckaers JHFM, **ten Brinck RM**, *et al.* Invloed van mictiehouding op urodynamische parameters bij mannen: een literatuuronderzoek. *Tijdschr Voor Urol* 2014;**4**:36–42. doi:10.1007/s13629-014-0008-5

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