Differences and similarities of autoantibody-positive and autoantibody-negative rheumatoid arthritis during the disease course: on our way to personalized medicine

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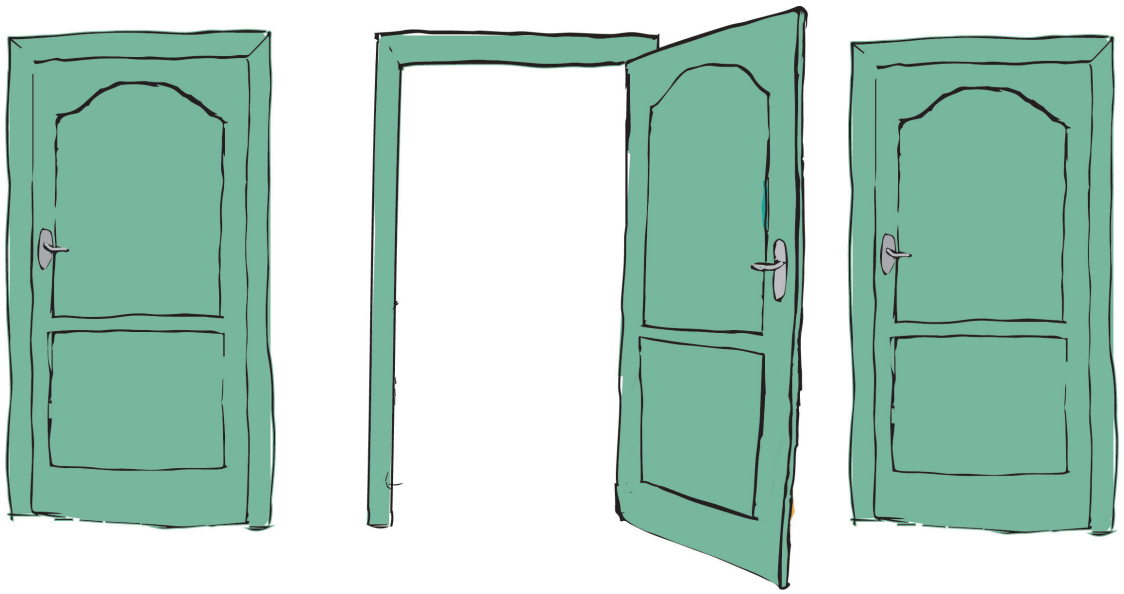
CHAPTER 1

Introduction



RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease. RA is predominantly characterized by inflammation of the small joints and often persists for a lifetime.[1] RA affects around 0.5% to 1% of the worldwide population, and every year, over 1 million patients are newly diagnosed with RA.[2] RA is more common in females than males (ratio 3:1), presents most often around the sixth decade of life and is more prevalent in western countries.[2]

Autoantibodies

Around 50% of RA patients have autoantibodies, such as anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor (RF). These autoantibodies often co-occur and are rare in the general population.[3-5] RA-patients are often classified as either being autoantibody-positive (with autoantibodies) or autoantibody-negative (without autoantibodies), because these autoantibodies are influential during the whole disease course: they are already present before the first complaints,[6] generally persist throughout disease [7] and associate with a more severe disease course.[8]

Clinical presentation

Both autoantibody-positive and autoantibody-negative RA patients typically present similarly with symmetrical swelling, tenderness and morning stiffness of the hands and feet' small joints, accompanied by systemic complaints such as fatigue, malaise and weight loss.[8] However, the first clinical presentation of RA might be very heterogeneous. The diagnosis is often made based on the treating rheumatologist's judgment and expertise based on "pattern recognition".

Classification criteria

For research purposes in general and clinical trials in particular, classification criteria for RA were derived to identify a homogeneous group of RA patients. 1987 ACR criteria were designed to optimally discriminate between patients with RA and patients with other rheumatological diseases.[9] Since the 1987 ACR criteria include late RA manifestations such as rheumatoid nodules and radiographic changes, they have low sensitivity for early RA patients (Table 1).

To identify patients with very early disease, the 2010 ACR/EULAR criteria have been developed (Table 1).[10] These criteria were designed to identify patients with persistent and/or erosive disease in an early stage. Since autoantibodies are present in early disease and strongly associated with both persistent and erosive disease, the 2010 ACR/EULAR criteria heavily lean on the presence of autoantibodies. Consequently, the 2010 ACR/EULAR criteria are very sensitive for very early autoantibody-positive RA. In contrast, the fulfillment of these criteria requires > 10 affected joints in autoantibody-negative patients.[11] The disparity in number of joints needed for classification caused unintended differences between autoantibody-negative and autoantibody-positive patients identified by the 2010 criteria.

Table 1. Classification criteria for Rheumatoid Arthritis

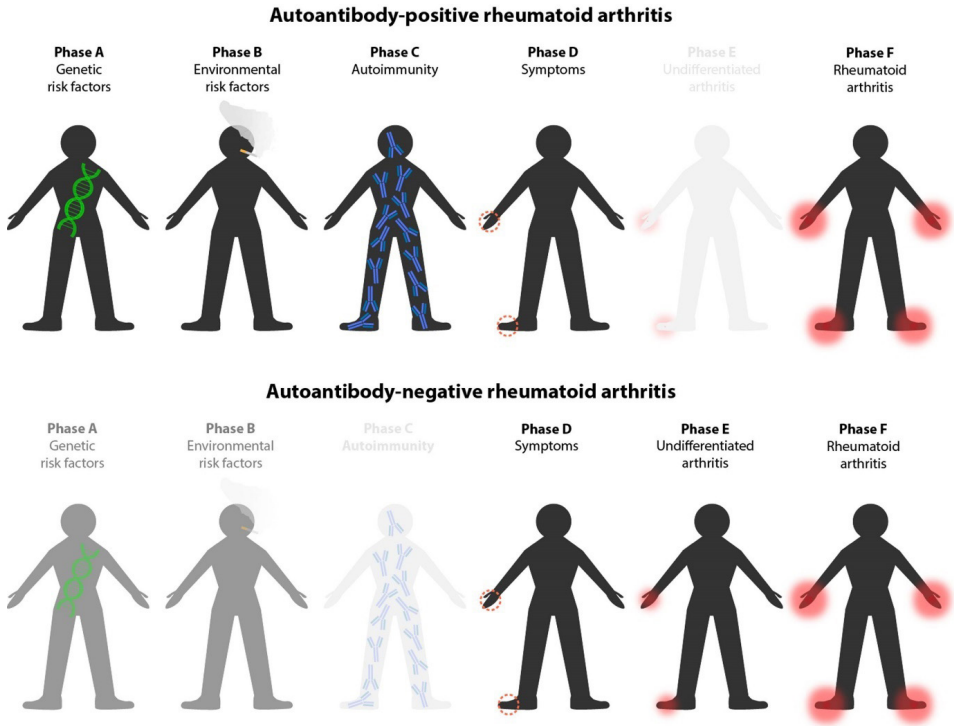
Revised ACR 1987 criteria	2010 ACR/EULAR criteria
Arthritis ≥3 joint areas (1)	Joint involvement
Arthritis of hand joints (1)	1 large joint (0)
Symmetric arthritis (1)	2–10 large joints (1)
Morning stiffness ≥ 1 hour (1)	1–3 small joints (2)
Rheumatoid nodules (1)	4–10 small joints (3)
Radiographic changes (erosions) (1)	>10 joints (5)
Presence of RF (1)	Serology
	ACPA and RF negative (0)
	Low-positive ACPA or RF (2)
	High-positive ACPA or RF (3)
	Acute-phase reactants
	Normal CRP and ESR (0)
	Abnormal CRP or ESR (1)
	Duration of symptoms
	<6 weeks (0)
	≥6 weeks (1)
4/7 criteria must be present to fulfil criteria	6/10 points must be present to fulfil criteria

Legend: ACR: American college of rheumatology; EULAR: European league against rheumatism; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibodies; CRP: c-reactive protein; ESR: erythrocyte sedimentation rate.

RA BEFORE DIAGNOSIS

The diagnosis with either autoantibody-positive or autoantibody-negative RA is often preceded by a preclinical phase with systemic alterations, arthralgia and (in case of autoantibody-positive RA) presence of autoantibodies.[6,12] The EULAR study group for risk factors for RA formulated terminology for future research.[13] The working group formulated six phases: (A) genetic risk factors for RA, (B) environmental risk factors, (C) systemic autoimmunity, (D) symptoms without clinical arthritis, (E) unclassified arthritis and (F) rheumatoid arthritis. However, not all phases are (equally) applicable to both autoantibody-positive and autoantibody-negative disease as displayed in Figure 1 and elaborated on below.

Figure 1. The phases of RA as defined by the EULAR study group for risk factors for RA in autoantibody-positive and autoantibody-negative RA



Legend: The shade of the phase represents the frequency of identification and/or presence of that phase to autoantibody-positive and autoantibody-negative rheumatoid arthritis, respectively. Black phases are often identified, grey phases are less often identified than in the other autoantibody group and light grey phases are (almost) never identified.

Phase (A): genetic risk factors

The overall heritability of RA is estimated to be ~40%. However, "genetic risk factors" are predominantly identified in autoantibody-positive RA and seem to play a more prominent role in this RA type. The best described genetic risk factor for RA, the HLA shared epitope, is associated with RA only in ACPA positive patients.[14,15] Moreover, autoantibody-positive RA has a higher heritability [16]. Genome Wide Association Studies (GWAS) have identified small frequent variations in the human genome, Single Nucleotide Polymorphisms (SNPs), that associate with RA risk. Similar to the HLA shared epitope, most SNPs only associate with the development of autoantibody-positive RA.[17] In contrast, extensive GWAS have failed to identify genetic risk factors for autoantibody-negative RA.[17] This difference between autoantibody-positive and autoantibody-negative RA might be explained by the heterogeneity of autoantibody-negative RA and misdiagnosis of autoantibody-negative RA patients. However, until now, no studies have supported the hypothesis that autoantibody-negative RA is more heterogeneous. Altogether, the genetic risk seems to be predominantly confined to autoantibody-positive RA.

Phase (B): environmental risk factors

Regarding phase B, "environmental risk factors", RA's most important risk factor is smoking.[18,19] Increasing evidence has stated that smoking is associated with mainly autoantibody-positive RA development.[19] Another environmental risk factor is microbiomic alterations, that also precede RA development. These microbiomic alterations are again mainly associated with the development of autoantibody-positive RA.[18] Here again, heterogeneity of the autoantibody-negative RA subtype might influence results and could have prohibited discovery of environmental risk factors. However, it seems that environmental risk plays a more prominent role in autoantibody-positive RA.

Phase (C): systemic autoimmunity

"systemic autoimmunity" is often defined as the presence of autoantibodies such as ACPA and RF. Autoantibody-negative patients might also have (undiscovered) autoantibodies. Many researchers have tried to close this so-called "serological gap" and discovered new autoantibodies such as AAPA and anti-Carp.[20] However, most patients with these autoantibodies already have ACPA and or RF, leaving the serological gap mostly unchanged.[21]

The absence of discovered autoantibodies might impose the idea that inflammation does not play a role in the pathogenesis of autoantibody-negative RA. However, this seems not to be the case as markers of systemic inflammation, such as erythrocyte sedimentation rate (ESR) or the C-reactive protein (CRP), are present and already increased compared to healthy controls before complaints both in autoantibody-negative and autoantibody-positive RA.[22,23]

Phase (D): symptoms without clinical arthritis

The fourth phase, symptoms without clinical arthritis, can be studied in risk groups of persons with a reasonable chance to develop RA. Two types of risk groups are often studied: autoantibody-positive persons with musculoskeletal complaints and clinically suspect arthralgia (CSA) patients. Since seropositivity generally persists, autoantibody-positive arthralgia patients usually convert to autoantibody-positive RA.

In contrast, clinically suspect arthralgia patients can be both autoantibody-positive and autoantibody-negative both at presentation and at the moment of arthritis development: At presentation, 14% of CSA patients are ACPA-positive whereas 45% of CSA patients who develop arthritis are ACPA-positive.[12,24] Because risk factors

for arthritis development might differ for autoantibody-positive and autoantibody-negative patients and the CSA cohort includes both autoantibody positive and autoantibody-negative patients, some identified risk factors might differ between strictly autoantibody-positive cohorts and the CSA cohort.

Phase (E): unclassified arthritis

The fifth phase, the phase of unclassified arthritis, is the phase in which arthritis is already present. Patients are suspect for RA but cannot be definitively diagnosed with RA yet. As mentioned before, the 2010 criteria were designed to classify patients as having RA in an earlier stage. However, the 2010-criteria also heavily load on the presence of autoantibodies. Therefore, patients with autoantibodies are classified as RA without many other factors present: they only need one small affected joint to be classified. In contrast, patients without autoantibodies need >10 affected joints to fulfill the 2010 criteria. Therefore, the remaining patients with unclassified arthritis are predominantly autoantibody negative and most autoantibody-positive patients apparently skip the phase of unclassified arthritis.

RA AT DIAGNOSIS AND DURING DISEASE

RA is a long-term, potentially invalidating disease. While RA predominantly affects the joints, it can also cause systemic symptoms such as fatigue, decreased functionality and extra-articular manifestations. On the societal scale, RA affects work productivity and participation. RA can cause excess mortality and RA treatment can cause increased healthcare costs. Therefore research on RA can focus on the joint level, the patient level and the societal level.

Joint-level

Clinical

Inflammation in RA at the joint level can lead to swelling of the joints, tenderness of the joint and movement impairment of the joint.[25] Because no objective measure of disease activity in RA exists, composite indices are used to measure disease activity in RA. These composite indices incorporate the number of swollen and the number of tender joints from a prespecified subset. Next to these joints scores, often a measure of patient evaluated global disease activity are incorporated.[26] Perhaps the most used example of a composite measure of disease activity is the disease activity score (DAS) and a simplification of the DAS that omits the joints in the feet (DAS28).[27,28] These composite measures also include objective systemic inflammatory markers (CRP, ESR). At baseline, the distribution of affected joints and DAS at baseline is similar for autoantibody-positive and autoantibody-negative RA patients.[8,29] Under randomized and protocolized treatment, DAS over time has been reported both to be lower and higher in autoantibody-positive and autoantibody-negative patients.[30,31]

Damage

Prolonged inflammation in joints can lead to permanent bone and cartilage damage in joints.[32,33] The Sharp - van der Heijde score is most often used to quantify bone and cartilage damage. This score incorporates erosions and joint space narrowing in the hands and the feet.[34] Damage can lead to disability.[35] Treating the DAS be below a certain threshold that we often call “remission” prevents long-term damage, for the most part, irrespective of medication used to keep remission.[36] Treating until remission is achieved is called the treat-to-target strategy. Drugs that are used to lower the DAS to in the end prevent damage are called disease modifying anti-rheumatic drugs (DMARDs). Autoantibody-positive patients have more damage progression over time.[8] Moreover, it has been hypothesized that the relationship between disease activity and joint damage is only present in autoantibody-positive RA.[32]

MRI + Ultrasound

While X-ray imaging can be used to detect damage in joints, X-ray images are not suitable to visualize inflammation. Ultrasound imaging and Magnetic Resonance Imaging (MRI) can be used to visualize both intraarticular inflammation such as synovitis as juxta-articular inflammation such as tenosynovitis.[37] MRI is more sensitive for the detection of these features.[37] Moreover, MRI has better reproducibility and MRI can visualize bone marrow edema that represents inflammation in the bone (osteitis).[38] MRI inflammation is predictive for arthritis development in both autoantibody-negative and autoantibody-positive arthralgia patients.[39] However, during progression to RA, the trajectories of MRI inflammation might differ between autoantibody-positive and autoantibody-negative RA. Therefore the optimal MRI predictors might differ between those subsets.[40] At arthritis diagnosis, the amount of synovitis and tenosynovitis is similar between autoantibody-positive and autoantibody-negative patients with UA or RA. In contrast, the amount of osteitis was higher in autoantibody-positive patients in this study.[41] However, comparisons of diagnosed RA patients are scarce and comparisons of the trajectories of MRI inflammation after arthritis between autoantibody-positive and autoantibody-negative RA patients were not performed yet.

Patient-level

Inflammation in the joint, damage of the joints and systemic inflammation can lead to disability and patient-reported disease-related complaints (PROMs) such as pain, fatigue and morning stiffness.[42,43] While inflammation contributes to these complaints, also other factors such as mental health and coping mechanisms contribute majorly to these complaints.[42]

Besides, RA is also related to a higher prevalence of stress and depression.[44] Stress and depression also predict higher disease activity over time.[44] Concludingly, inflammation, patient-reported complaints and mental health are factors that influence each other and strengthen each other. However, how and if this differs between autoantibody-positive and autoantibody-negative RA is insufficiently described. Depression is associated with subsequent development of autoantibody-negative RA but not autoantibody-positive RA.[45] Autoantibody-positive and autoantibody-negative RA patients have similar trajectories of functionality and PROMs over time.[46]

Societal impact

Epidemiology

Since RA often emerges in the sixth decade of life, the age distribution influences the incidence of RA. Since worldwide more persons reach the sixth decade, the incidence is rising.[2] Since autoantibody-negative RA patients tend to be older, it can be hypothesized that autoantibody-negative incidence will increase even more. However, until now, this remains to be elucidated.

The incidence of RA, the mortality of RA patients and the extent to which RA can be "cured" influence the prevalence of RA. RA patients experienced excess mortality compared to the general population. However, this excess mortality seems to have disappeared after the introduction of treat-to-target strategies.[36,47,48] Excess mortality in RA is predominantly caused by cardiovascular death. However, it is unknown whether this is true for both autoantibody-positive and autoantibody-negative RA.

Costs

The economic burden of RA depends on both the work productivity lost as well as the medication costs. The European medicine agency approved the first biological infliximab for RA treatment in the early 2000s.[49] Since biologicals are more expensive than other drugs, these contribute most to the medication costs of RA.[50] Altogether, ~80.000.000 euros are spent annually on the care of RA patients in the Netherlands. This number will probably rise by ~10.000.000 within the coming ten years.[51] Generally, autoantibody-positive RA more often requires biological treatment.[52] In contrast, work loss in RA is substantial and does not differ between autoantibody-positive and autoantibody-negative RA.[46]

CURE OF RA

While RA persists for a lifetime in most RA patients, some patients achieve sustained DMARD-free remission (SDFR). SDFR is the absence of any swollen joint after more than one year of DMARD cessation.[53] Therefore this is a clinical proxy for 'cure'. This outcome has become increasingly achievable since early methotrexate treatment is associated with normalization of functionality and low patient-reported symptoms.[54] The absence of autoantibodies and shared epitope alleles increased the chance of achieving DFR.[53]

AIMS

Concludingly, while autoantibody-positive and autoantibody-negative RA have similar clinical presentations, it has become increasingly clear that they also have many differences. Therefore it has been hypothesized that autoantibody-negative and autoantibody-positive RA are distinct diseases that require different diagnosis and treatment. However, this hypothesis has not been systematically studied.

Therefore this thesis aims to assess the differences and similarities between autoantibody-positive and autoantibody-negative RA from the start of complaints to the end of the disease.

The described research was performed with the ultimate goal to clarify whether autoantibody-negative and autoantibody-positive RA are distinct diseases that require different diagnoses and treatment.

COHORTS

CSA

To address these questions, we took advantage of two large observational cohorts based in the Leiden university medical center (LUMC): The CSA cohort and the early arthritis cohort (EAC). In the CSA-cohort patients are included that are at risk of RA development, according to their rheumatologist, that have not developed arthritis yet.[12] At baseline, rheumatologists and patients completed questionnaires, swollen and tender joint counts were performed and blood samples were taken for routine diagnostic laboratory screening. Besides, a unilateral 1.5 Tesla MRI of the MTP, MCP and wrist joints was performed. Patients were prospectively followed with scheduled visits at 4, 12 and 24 months; additional visits were scheduled in case of increasing symptoms.

EAC

Patients with recently developed arthritis were included in the Leiden EAC. This cohort

exists since 1993 and is extensively described elsewhere.[55] Also, in this cohort, patients received physical examinations, questionnaires and laboratory screening at baseline. In principle, patients are followed until discharge from the Rheumatology outpatient clinic. In 2010 MRI scans were added to the baseline visit, and until 2015 MRIs were repeated during the first two years.

The most prominent advantage of the Leiden EAC is that all rheumatologists in the Leiden area are affiliated to the LUMC and that inclusion in the Leiden EAC has been part of standard treatment for early arthritis patients since 1993. Moreover, inclusion criteria have not changed over time and all patients have been subjected to regular yearly follow-up visits by trained research nurses. This ensures a representative sample of arthritis patients with regularized measurements during follow-up.

OUTLINE

This thesis follows the disease course of a RA patient from start of complaints to the end of disease. Three phases are studied: The pre-arthritis phase from the beginning of complaints until arthritis development, the early arthritis phase from arthritis development until 2 years after and the long-term outcomes that were observed until 15 years after diagnosis.

Pre-arthritis

In **Chapter 2**, we studied the pre-arthritis phase and analyzed which combinations of MRI-features at presentation with CSA were predictive for RA-development to increase our comprehension of locations of RA-onset and improve the predictive accuracy of MRI in a cohort with both autoantibody-positive and autoantibody-negative patients.

Early arthritis

In **Chapter 3**, we studied early arthritis patients before treatment. We hypothesized that if MRI-detectable tenosynovitis is a true RA-feature, the sensitivity for RA is high at diagnosis, in both autoantibody-positive and autoantibody-negative RA, and lower in other diseases and investigated this in the EAC cohort. Again concerning early arthritis patients, in **Chapter 4** we determined trends in incidence of autoantibody-negative and autoantibody-positive RA around Leiden. We also examined how the age-distribution of the population affected this incidence and what this would implicate in the future. In **Chapter 5**, we studied the association of fatigue and MRI inflammation at diagnosis and during the first two years of disease course in a large consecutive cohort of >500 RA patients and stratified for autoantibody status. In **Chapter 6**, the time order of inflammation decrease after treatment was investigated in early UA and RA patients and compared between autoantibody-positive and autoantibody-negative patients

Long-term outcomes

In **Chapter 7,** we studied the changes in disease activity and three long term outcomes (functionality, mortality and SDFR) in autoantibody-negative and autoantibody-positive RA patients over the last 25 years. Because similar RA patients presented over these years and treatment changed, we could assess the influence of treatment on these long-term outcomes by using inclusion period as a proxy for treatment strategy. Finally, in **Chapter 8**, the impact of improved treatment on population corrected mortality was studied in both autoantibody-negative and autoantibody-positive RA.

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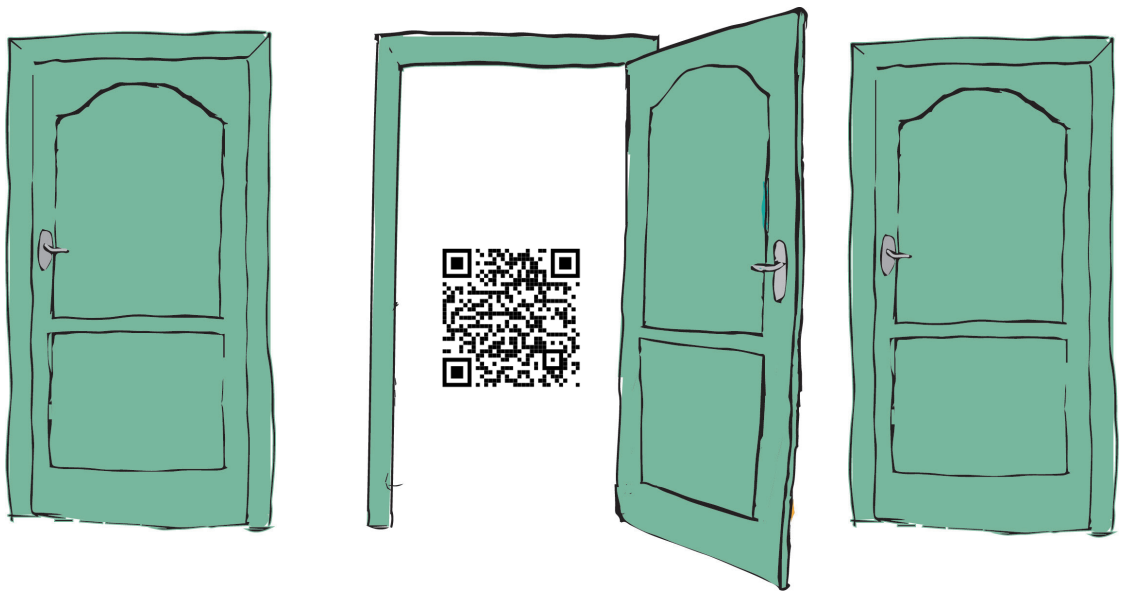
PRE-ARTHRITIS



CHAPTER 2

A search to the target tissue in which RA-specific inflammation starts: A detailed MRI study to improve identification of RA-specific features in the phase of Clinically Suspect Arthralgia

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ABSTRACT

Objective

Based on a unique cohort of clinically suspect arthralgia (CSA) patients, we analysed which combinations of MRI-features at onset were predictive for Rheumatoid Arthritis (RA) development. This was done to increase our comprehension of locations of RA-onset and improve the predictive accuracy of MRI in CSA.

Methods

In the discovery cohort, 225 CSA-patients were followed on clinical arthritis development. Contrast-enhanced 1.5T MRIs were made of unilateral MCP(2-5), wrist and MTP(1-5)-joints at baseline and scored for synovitis, tenosynovitis and bone marrow edema. Severity, number and combinations of locations (joint/tendon/bone) with subclinical inflammation were determined, with symptom-free controls of similar age category as reference. Cox regression was used for predictor selection. Predictive values were determined at 1-year follow-up. Results were validated in 209 CSA-patients.

Results

In both cohorts 15% developed arthritis <1-year. The multivariable Cox model selected presence of MCP-extensor peritendinitis (HR 4.38 (2.07-9.25)) and the number of locations with subclinical inflammation (1-2 locations HR 2.54 (1.11-5.82); ≥3 locations HR 3.75 (1.49-9.48)) as predictors. Severity and combinations of inflammatory lesions were not selected. Based on these variables, five risk-categories were defined: no subclinical inflammation, 1-2 or ≥3 locations, with or without MCP-extensor peritendinitis. Positive predictive values (PPVs) ranged 5% (lowest category; NPV 95%)-67%(highest category). Similar findings were obtained in the validation cohort; PPVs ranged 4% (lowest category; NPV 96%)-63%(highest category).

Conclusion

Tenosynovitis, particularly MCP-extensor peritendinitis, is among the first tissues affected by RA. Incorporating this feature and number of locations with subclinical inflammation improved prediction making with PPVs up to 63-67%.

BACKGROUND

Since a decade increasing attention is being paid to identify patients in 'pre-rheumatoid arthritis' stages, among which the symptomatic stage preceding clinical arthritis. This is done with the assumption that earlier identification of patients with (imminent) rheumatoid arthritis (RA), allows earlier intervention and thereby may result in better disease outcomes. This hypothesis is being evaluated in several ongoing proof of concept trials [1-4]. Currently, accurate risk stratification is crucial to include patients at high risk to enhance the power of these trials [5]; in the future it might be valuable to prevent overtreatment as much as possible.

Risk stratification is optimal if both positive and negative predictive values (PPV, NPV) are high. Importantly, both values strongly depend on prior risks. The prior risk of developing arthritis in at risk populations, either asymptomatic, such as healthy relatives of patients with RA, or symptomatic, is relatively low [6,7]. Consequently, any test that is applied in an at risk population easily reaches a high NPV but PPVs generally remain low. Patients with Clinically Suspect Arthralgia (CSA) are considered to be at risk for progression to RA based on the clinical presentation according to their rheumatologists. Only ~8% of patients presenting with arthralgia at rheumatologic outpatient clinics are identified as having CSA and these patients have, compared to the other arthralgia patients, a 55 times increased odds to develop RA [7]. This shows the accuracy of clinical expertise as first discriminator. Nonetheless, without further risk stratification, the absolute risk on RA development in this population is still moderate (~20%) [8]. Hence, other biomarkers are needed in patients with CSA to achieve accurate prediction making and high PPVs in particular.

Different type of biomarkers have been studied, among which auto-antibodies, markers of systemic inflammation and subclinical joint inflammation [9,10]. The presence of imaging-detected subclinical inflammation in hand and foot joints has been shown predictive for progression to RA in several studies, both when using Ultrasound (US) or Magnetic Resonance Imaging (MRI) [6,8,11]. Although less accessible, MRI has the advantages that it can depict bone marrow edema (BME) and is more sensitive and reproducible than US [12]. Previous studies have revealed that some degree of MRI-detected inflammation is also present in symptom-free persons of the general population, especially at higher age [13,14]. The nature of these features is not completely elucidated and degeneration may explain part of these findings. However, for diagnostic and prognostic purposes it has been evidently shown that using asymptomatic persons as reference when defining a positive MRI decreased the number of false-positive results and increased the specificity and predictive accuracy of MRI [15]. We previously observed that patients with CSA and a positive MRI, i.e.

inflammation more than this reference, have a risk of 31% to progress to RA during the next year. The NPV of a negative MRI was high (94%) [8].

Thus far, the predictive accuracy of MRI-detected subclinical inflammation in CSA has not been validated. Moreover, we hypothesized that presence of certain inflammatory MRI-features could be associated with a higher risk on RA development. We therefore aimed to determine if the PPV of MRI can be improved by not only evaluating the presence of subclinical inflammation but also incorporating information on the severity, the number and combinations of affected locations. We also aimed to validate the predictive accuracy of MRI in a separate set of patients with CSA. Finally, detailed studies on MRI predictors might also increase our understanding of the joint tissues that are first affected during RA development.

METHODS

Patients

All patients studied were included in the Leiden CSA-cohort, which has been described elsewhere [16]. In short, CSA-patients had recent-onset (<1 year) arthralgia of hand or foot joints and were considered at risk for progression to RA based on the clinical expertise of the rheumatologist. Per definition CSA was not present if patients presented with clinical arthritis or if another explanation for the symptoms (e.g. osteoarthritis, fibromyalgia) was more likely. Furthermore, auto-antibodies were rarely determined in primary care, in line with Dutch GP-guidelines [17]. Hence inclusion was mainly based on the clinical expertise (including pattern recognition) of rheumatologists. We have previously shown that the expertise of the rheumatologist is valuable in differentiating arthralgia patients [7].

The Leiden rheumatology outpatient clinic has close contact with GPs and early referral clinics to allow access to secondary care without delay [18]. This provided an unique setting to identify patients with joint symptoms at risk for RA development before clinical arthritis has developed. From all patients newly presenting with arthralgia, only a small percentage is identified as having CSA by rheumatologists [7]. Notably, the cohort was founded before the development of the EULAR definition of arthralgia suspicious for progression to RA and fulfilment of this definition was not mandatory. MRI was made at baseline. Patients were prospectively followed with scheduled visits at 4, 12 and 24 months; additional visits were scheduled in case of increasing symptoms [16].

The Leiden CSA cohort was split in two data-sets. Between April 2012-April 2015 241 patients with CSA were consecutively included; of these 225 had a baseline MRI and were studied as discovery cohort. CSA-patients presenting between April 2015-September 2017 were evaluated for validation (n=298). Patients that participated in a randomized double-blind proof-of-concept trial (50% treated with methotrexate, 50% with placebo) (n=73) and patients without a MRI (n=16) were excluded from the validation data-set (see Flow-chart Supplementary file 1). Hence, 209 CSA-patients were studied for validation; Baseline characteristics (age, sex, symptom duration, number of painful joints, CRP, auto-antibody status) did not differ between patients with and without MRI (Supplementary file 2). Participation in the trial required presence of MRI-detected subclinical inflammation. There were no differences in baseline characteristics between eligible patients with subclinical inflammation that were included in the validation cohort and were excluded because of trial participation (Supplementary file 3).

MRI

MRI with a musculoskeletal (MSK)-extreme 1.5 Tesla (T) MRI-scanner (GE, Wisconsin, USA) was performed at baseline of metacarpophalangeal (MCP(2-5)), the wrist, and metatarsophalangeal (MTP(1-5))-joints on the most painful side (dominant side in case of symmetric symptoms) <1-week after the first visit to the outpatient clinic. A detailed scan and scoring protocol is provided in Supplementary file 4. MRIs were scored in line with RAMRIS by two readers blinded to clinical data [19,20]. The interreader and intrareader ICCs were all >0.90 (Supplementary file 5).

As done previously, an MRI was considered 'positive' when subclinical inflammation was present; meaning both readers scored inflammation (synovitis, BME or tenosynovitis) in ≥1 location that was present in <5% of the healthy persons in the same age-category at the same location [13,15,21]. Thus, since inflammation is scored semi-quantitively, it must be 1 RAMRIS-point above the 95th percentile of healthy individuals of the same age-group. Reference values were obtained from previous research in which we scanned 193 healthy volunteers of three age-categories [13].

Patients and rheumatologists were blinded to all MRI-data in the discovery cohort. In the validation cohort, presence/absence of MRI-positivity was disclosed (because it determined eligibility for a double-blind proof-of-concept trial) but patients and rheumatologists remained blinded for any further detailed MRI-data (such as on specific MRI-features or locations).

Outcome

The main outcome was development of clinically apparent inflammatory arthritis, objectified at physical examination by rheumatologists. None of the patients used DMARDs (including glucocorticoids) before arthritis development. The secondary

outcome was development of RA, defined as clinical diagnosis plus fulfilment the 1987 or the 2010 criteria for RA (ACPA-negative patients with diagnosis of RA have difficulties fulfilling the criteria as ≥11 involved joints are required, whereas ACPA-positive patients can fulfil the criteria with only 1 swollen joint [22-25]; to prevent a possible bias for ACPA-negative patients, patients that fulfilled the 1987 criteria were also classified as RA).

Statistical Analyses

MRI-features studied to identify predictors

We aimed to investigate the severity, the number and combinations of locations with subclinical inflammation. These MRI-features were defined/selected as follows:

Severity: Severe subclinical inflammation was defined as 2 RAMRIS-points scored by both readers above the reference described above.

Number of locations with subclinical inflammation: The number of locations (joint/bone/tendon) was counted and categorized after visual inspection of Kaplan Meijer curves.

Combinations of types and locations: Since incorporating all possible combinations of lesions in standard analysis would cause significant risk of overfitting, we implemented three methods to search for potentially predictive combinations: Firstly, all possible pairs of MRI-features were plotted and coloured according to their prevalence in converters and non-converters (no clinical arthritis development <1-year); combinations that were visually potentially predictive were selected. Because presentation of raw data presentation is insightful, but also has disadvantages, all possible pairs of inflammatory MRI-lesions were also studied with least absolute shrinkage and selection operator (LASSO) regression (lambda minimizing the 10-fold cross-validation error) [26]. Finally, principal component analysis (PCA), incorporating all inflammatory MRI-features, was performed to find potentially predictive combinations composed of multiple MRI-features. The first two components were considered as potential predictors.

Model derivation

Kaplan Meier curves and univariable Cox regression were used to study the candidate MRI-variables with time until arthritis development as outcome. Significant predictors (<0.05) were checked for collinearity with Pearson correlations (<0.7), before performing multivariable Cox analyses. All candidate predictors were entered in the model and backward selection was performed (p<0.10). To confirm the selection of predictors we also added the predictors in a LASSO regression model and studied how often they remained in the model in 1000 bootstrap replications [26]. Risk groups were made based on the identified predictors and the observed 1-year risk of developing inflammatory arthritis was calculated in each of the risk groups with logistic regression. In these analyses 1-year follow-up data were used; thus patients that developed clinical arthritis after year-1 were categorized as non-convertors. Five patients (2.2%) were lost to follow-up in year-1 and considered as non-convertors. PPVs, NPVs and area under the curve (AUC) were determined. Calibration was assessed with the Hosmer-Lemeshow test and a calibration graph.

Validation

We used the model of the discovery cohort to predict the one year survival probabilities of the individuals in the validation cohort and validated the PPVs in the validation cohort. Calibration and predictive values were assessed similar to the discovery cohort. Eight patients (3.8%) were lost to follow-up in the first year and considered as non-convertors.

Patients in the validation cohort with a positive MRI who participated in a randomized double-blind trial were excluded. Exclusion of part of eligible patients with a positive MRI (which is associated with arthritis development) could affect the rate of arthritis development in the validation cohort. We therefore accounted for MRI-positivity by including the number of locations (0=Negative MRI; 1-2/≥3= positive MRI) in all multivariable models. Other characteristics of the patients with subclinical inflammation that were included and excluded from the validation cohort were similar (Supplementary file 3), therefore adjustment for MRI positivity is sufficient to adjust for the lower number of patients with positive MRI in the validation set. This is extensively explained in Supplementary file 6.

Sensitivity analyses

Predictive values were verified with the outcome inflammatory arthritis after two years in patients that were included 2 years before data extraction.

Also, predictive values were assessed in the subgroup of CSA-patients that also fulfilled the EULAR-definition of arthralgia suspicious for progression to RA, as this is a more homogeneous subset of patients, with a slightly higher risk for RA [27,28].

Predictive values were also assessed for the secondary outcome, development of RA. Analysis were performed using SPSS 23 and R 3.5.0. P-values <0.05 were considered significant.

RESULTS

Baseline characteristics

Baseline characteristics are shown in Table 1. Characteristics of both cohorts were similar, except for a lower frequency of MRI-positivity in the validation cohort (51% versus 35%; p=0.002).

Discovery cohort

Within a median follow-up of 108 weeks (IQR 54-114) 42 patients progressed to clinical arthritis, and 34 (15%) did so within the first year.

Identification of predictors

In univariable analysis, severe subclinical inflammation was predictive for inflammatory arthritis development (Table 2).

Table 1: Baseline clinical and MRI characteristics of patients included in the discovery and validation cohorts

	Discovery cohort (n=225)	Validation cohort (n=209)	p-value
Age in years, mean (SD)	44 (13)	43 (12)	0.26
Female, n (%)	174 (77)	165 (79)	0.77
Symptom duration in weeks, med (IQR)	17 (9-32)	20 (9-44)	0.28
Localisation of initial symptoms			0.39
Small joints, n (%)	189 (84)	165 (79)	
Small and large joints, n (%)	22 (10)	26 (13)	
Large joints n(%)	13 (6)	17 (8)	
Localisation of initial symptoms			0.76
Upper extremities, n (%)	162 (72)	134 (70)	
Upper and lower extremities, n (%)	39 (17)	34 (18)	
Lower extremities, n (%)	23 (10)	24 (13)	
Symmetrical localisation of initial symptoms, n (%)	166 (74)	127 (70)	0.35
Morning stiffness ≥ 60 min, n (%)	72 (36)	62 (34)	0.83
68-TJC, med (IQR)	6 (3-10)	5 (2-10)	0.23
Fulfilling the EULAR definition of CSA, n (%)	153 (68)	131 (63)	0.29
CRP-level in mg/L, med (IQR)	3 (3-5)	3 (3-4)	0.59
ESR-level in mg/L, med (IQR)	6 (2-13)	6 (2-14)	0.12
RF, n (%)	46 (20)	41 (20)	0.92
ACPA, n (%)	28 (12)	30 (14)	0.66
MRI-detected presence of subclinical inflammation (MRI-positivity), n (%)	114 (51)	74 (35)	0.002

Legend: p-value: Chi-square tests, Fishers's exact tests, Student's t-tests and Wilcoxon's rank sum tests were applied as appropriately. SD: Standard deviation; n:number of patients; RA: Rheumatoid arthritis; med: median; IQR: interquartile range; EULAR: European league against rheumatism; CSA: Clinically suspect arthralgia; BME: Bone marrow edema; min: minutes; TJC: Tender joint count; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; RF: Rheumatoid factor; ACPA: Anti-citrullinated protein antibody; MRI: Magnetic Resonance Imaging

Table 2: Results of univariable and multivariable Cox regression in discovery cohort with clinically apparent inflammatory arthritis as outcome.

	Univariable	Final modelafter backward selection
Number of locations with subclinical inflammation		
0 locations (negative MRI)	Ref	Ref
1 or 2 locations	3.14 (1.40-7.04)	2.54 (1.11-5.82)
3 or more locations	6.28 (2.77-14.2)	3.75 (1.49-9.48)
Severe subclinical inflammation*	3.34 (1.48-7.54)	-
MCP-extensor peritendinitis	7.85 (3.91-15.8)	4.38 (2.07-9.25)
Combination of inflammatory lesion in wrist and MTPs	2.19 (1.15-4.16)	-
PCA-component 1	0.92 (0.88-0.96)	-
PCA-component 2	0.93 (0.83-1.04)	-

Legend: *Severe subclinical inflammation: Inflammation that is 2 RAMRIS-points above the 95th percentile of inflammation observed in healthy volunteers in the same age-category as published previously [13]. Further explanation in Supplementary file 4.
MCP: metacarpophalangeal; MTP: metatarsophalangeal; n = number of patients

With respect to the number of locations with subclinical inflammation. Visual examination of Kaplan Meier analysis resulted in three subcategories: 0 locations with subclinical inflammation, 1-2 locations and ≥3 locations (Supplementary file 7). As shown in Table 2, the number of locations was predictive for arthritis development.

Prevalence of all pairs of MRI-features were plotted for patients with and without arthritis development ≤1-year (Figure 1). Visual inspection suggested that a combination of inflammation in the wrist and in MTP-joints was predictive for arthritis development. Additionally all combinations with MCP-extensor peritendinitis, basically the presence of MCP-extensor peritendinitis, was potentially predictive. Therefore the combination of inflammation in the wrist and in MTP-joints, and the presence of MCP-extensor peritendinitis were studied further. Both variables were indeed significant in univariable Cox regression (Table 2; Supplementary file 7).

LASSO regression using all possible pairs of inflammatory MRI-lesions identified pairs that were very specific but present in few patients. Because most of these pairs were incorporated in the combination of wrist and MTP-inflammation and MCP-extensor peritendinitis (Supplementary file 8), these latter were used in further analyses.

PCA was performed to search for patterns composed of multiple MRI-lesions; this revealed no evident discrimination of patients with and without arthritis development. PCA-component 1 was predictive for arthritis development and PCA-component 2 was not (Table 2; Supplementary file 9).

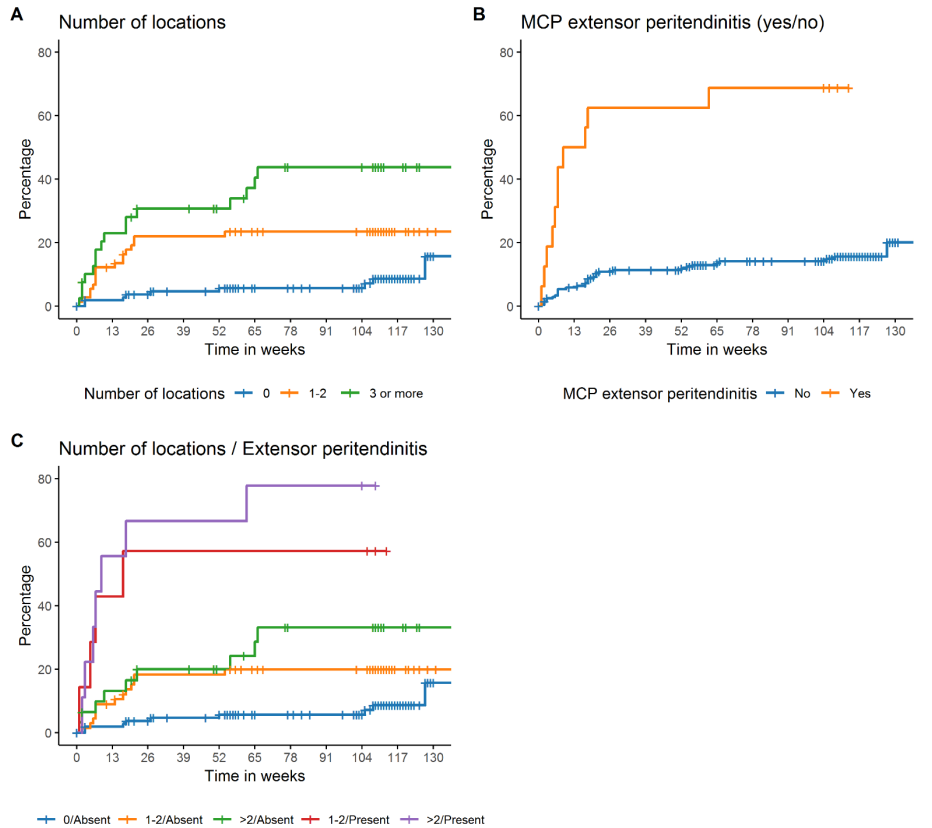
Figure 1: Plot of prevalence of all possible pairs of MRI inflammatory features in both converters and non-converters in the discovery cohort.

Legend: Pairs of features that were only present in patients that progressed to arthritis <1-year (converters; n=34) and not in non-convertors (n=191) are indicated in red. Pairs of features only present in non-convertors are indicated in green. The L-shaped box depicts extensor peritendinitis of the MCP(2-5) joints and the rectangle depicts a combination of inflammation (synovitis, tenosynovitis or BME) in the wrist and in MTP(1-5) MRI: Magnetic resonance imaging; CSA: Clinically suspect artralgia; BME: Bone marrow edema; MTP: metatarsophalangeal; MCP: metacarpophalangeal; HA: Hamate; CA: Capitate; TD: Trapezoid; TM; Trapezium; PI: Pisiform; TQ: Triquetrum; LU: Lunate; SC: Scaphoïd; UL: Distal ulna; RAD: Distal radius; Tenosynovitis Wrist: (I) extensor pollicis brevis, abductor pollicis longus; (II) extensor carpi radialis brevis, extensor carpi radialis longus; (III) extensor pollicis longus; (IV) extensor digitorum communis, extensor indicus proprius; (V) extensor digiti quinti proprius; (VI) extensor carpi ulnaris; (1) flexor carpi ulnaris; (2) ulnar bursa, including flexor digitorum profundus and superficialis tendon quartets; (3) flexor pollicis longus in radial bursa; (4) flexor carpi radialis.

Model derivation

Multivariable Cox regression of the five predictors revealed that number of locations and MCP-extensor peritendinitis were independently predictive, in contrast to severe subclinical inflammation, combination of an inflammatory lesion in wrist and MTPs and PCA-component 1 (Figure 2; Table 2). LASSO regression in 1000 bootstrapped datasets confirmed that the number of locations (1-2 locations 47%; ≥3 61%) and MCP-extensor peritendinitis (91%) were selected more often than severe subclinical inflammation (45%), the combination of an inflammatory lesion in wrist and MTP-joints (43%) and PCA-component 1 (53%).

Figure 2: Kaplan Meijer curves showing the associations with inflammatory arthritis development for the number of locations with subclinical inflammation (A), presence of MCP extensor peritendinitis (B) and both variables combined (C).



Legend:

1: 0/Absent: 0 locations with subclinical inflammation; No MCP extensor peritendinitis
2: 1-2/Absent: 1-2 locations with subclinical inflammation; No MCP extensor peritendinitis
3: >2/Absent: 3 or more locations with subclinical inflammation; No MCP extensor peritendinitis
4: 1-2/Present: 1-2 locations with subclinical inflammation; MCP extensor peritendinitis
5: >2/ Present: 3 or more locations with subclinical inflammation; MCP extensor peritendinitis

Based on the identified variables, patients were divided into five risk-groups: no subclinical inflammation ('negative MRI'), 1-2 and ≥3 locations of subclinical inflammation without MCP-extensor peritendinitis, 1-2 and ≥3 locations with MCP-extensor peritendinitis. A form to calculate this risk score is presented in Supplementary file 10 and online [29]. Logistic regression predicted PPVs of arthritis development in the five risk categories of: 5%, 18%, 20%, 60% and 64%, respectively. The observed PPVs were: 5%, 18%, 19%, 57%, and 67%, respectively. The NPV of no subclinical inflammation was 95% (Figure 3). Predicted and observed conversion rates were plotted in a calibration graph (Supplementary file 11); The Hosmer-Lemeshow test showed good calibration (p=0.92). The AUC was 0.74 (95% Confidence Interval 0.65-0.84). For comparison, a model that only considered MRI-positivity/MRI-negativity had an AUC of 0.69 (0.60-0.78) (Supplementary file 12).

Validation

At 1-year 15% (31/209) had developed arthritis. We validated the PPVs; the observed PPVs for arthritis development ≤1-year of the five risk-categories were 4% (lowest risk category), 19%, 59%, 50%, and 63% (highest risk category) respectively (Figure 3). The NPV of no subclinical inflammation was 96%. The AUC in the validation cohort was 0.81 (0.72-0.90) (Supplementary file 12).

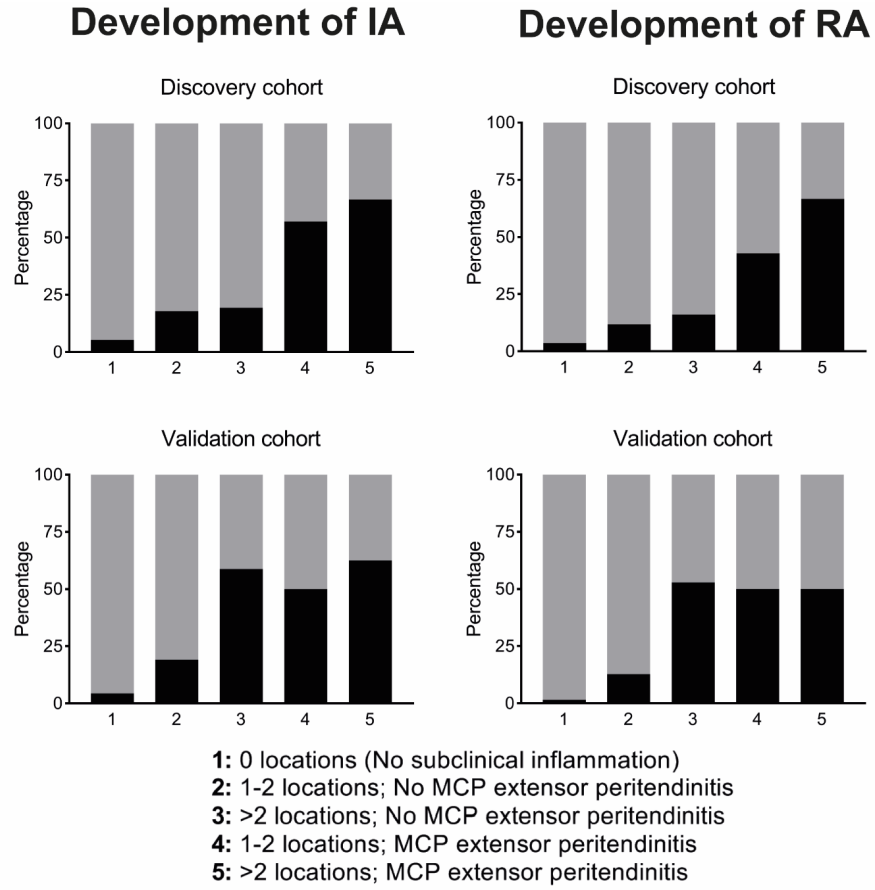
The calibration plot (Supplementary file 11) shows good calibration, except in the group with ≥3 locations without MCP-extensor peritendinitis (Predicted:20%, Observed:59%, n=17), yielding a significant Hosmer-Lemeshow test (p=0.01).

Sensitivity analyses

Predictive values were verified with the outcome inflammatory arthritis after 2 years follow-up. Slightly higher positive predictive values were obtained (Supplementary file 13).

Similar predictive values were obtained in the subgroup of CSA-patients that also fulfilled the EULAR definition (discovery, n=153; validation, n=131, Supplementary file 14). Also similar findings were obtained for RA-development as outcome.

Figure 3: Observed proportion of patients that developed clinical apparent inflammatory arthritis and rheumatoid arthritis in the first year (PPVs in black) per risk category in the discovery and validation cohorts.



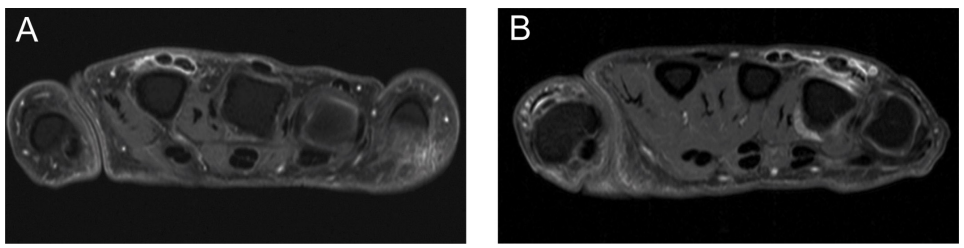
Legend: IA: clinically apparent Inflammatory Arthritis; RA: rheumatoid arthritis; locations: number of locations with subclinical inflammation.
Upper left graph: Positive predictive values on IA in the discovery cohort; No subclinical inflammation (5% (95% Confidence interval 3%-11%, n=111), 1-2 locations (18% (11%-29%), n=67) or ≥3 locations (19% (9%-36%), n=31) with subclinical inflammation but without MCP-extensor peritendinitis; and 1-2 locations (57% (25%-84%), n=7) or ≥3 locations (67% (35%-88%), n=9) with MCP-extensor peritendinitis.
Upper right graph: Positive predictive values on RA in the discovery cohort; No subclinical inflammation (4% (95% C.I. 1%-9%, n=111), 1-2 locations (12% (6%-22%), n=67) or ≥3 locations (16% (7%-33%), n=31) with subclinical inflammation but without MCP-extensor peritendinitis; and 1-2 locations (43% (16%-75%), n=7) or ≥3 locations (67% (35%-88%),n=9) with MCP-extensor peritendinitis.
Lower left graph: Positive predictive values on IA in the validation cohort; No subclinical inflammation (4% (95% Confidence interval 2%-9%, n=135), 1-2 locations (19% (10%-33%), n=47) or ≥3 locations (59% (35%-78%), n=17) with subclinical inflammation but without MCP-extensor peritendinitis; and 1-2 locations (50% (3%-97%), n=2) or ≥3 locations (63% (31%-86%), n=8) with MCP-extensor peritendinitis.
Lower right graph: Positive predictive values on RA in the validation cohort; No subclinical inflammation (1% (95% C.I. 0%-5%, n=135), 1-2 locations (13% (6%-25%), n=47) or ≥3 locations (53% (31%-74%), n=17) with subclinical inflammation but without MCP-extensor peritendinitis; and 1-2 locations (50% (3%-97%), n=2) or ≥3 locations (50% (22%-78%),n=8) with MCP-extensor peritendinitis.

DISCUSSION

We aimed to increase the understanding of the tissues that are already subclinically inflamed preceding the development of clinical arthritis and observed that MCP-extensor peritendinitis an early feature of RA. Moreover we aimed to optimize the predictive value of information provided by MRI for clinical arthritis and RA development in patients presenting in secondary care with CSA. MCP-extensor peritendinitis and the number of locations with subclinical inflammation and were independently predictive. Risk prediction of patients with a positive MRI was differentiated using these variables. Whereas patients with a positive MRI had, at group level, a PPV of 31% to develop RA during the next year [8], now a subgroup was found with a slightly lower risk (18-19%), but also subgroups with higher PPVs (up to 67%). The high NPV that was also observed previously was validated [8]. Importantly, this is the first study on the predictive accuracy of MRI in arthralgia that also demonstrated replication.

We observed that MCP extensor peritendinitis (see Figure 4 for an example) characteristically occurs before the development of clinical arthritis, in part of the RA-patients. MCP extensor peritendinitis is a relatively novel imaging finding, although several previous studied within classified RA showed that peritenditis of the MCP-extensors (visualized by MRI or US) has a high specificity for RA [28,29]. Whether involvement of this tendon occurs before or after other signs of inflammation (synovitis, osteitis) is unsolved, as longitudinal imaging data in the pre-arthritis phase of RA is scarce. Results of a recent study suggested that tenosynovitis of small joints in general was already increased at presentation with CSA, and preceded the development of osteitis and clinical arthritis, but further serial MRI studies are needed [30]. Whether micro-channels in the bare area of the joint are important in the spreading of inflammation is also a subject for further investigations.

Figure 4: MRI examples of MCP extensor peritendinitis



Legend: MCP extensor peritendinitis in two CSA patients, depicted in T1-weighted FSE sequences with frequency selective fat saturation in the axial plane of the MCP joints after injection of gadolinium contrast. Patient A had extensor peritendinitis at the level of MCP 2. Patient B had extensor peritendinitis at MCP 4; this patient also had peritendinitis at the level of MCP 3 and synovitis at MCP 4 that was better visualized at adjacent slices.

The plantar side of the hand has been studied anatomically and a tendon sheath at the level of MCP-joints was found. The extensor side, however, is less extensively studied, but a tendon sheath here has not been documented evidently [31]. Therefore the nature of the signal around the extensor tendons at the MCPs is as of yet unclear and is an interesting subject for further studies.

No validated scoring methods for MCP extensor peritenditis exist, therefore we adopted the method as proposed by Haavaardsholm et al. [19]. Now the relevance of this MRI-finding has been shown, further development and validation of scoring methods is warranted.

This study made more efficient use of the information obtained by MRI. Nonetheless and not unexpectedly, the accuracy of MRI alone was moderate and can presumably be improved by adding other biomarkers (e.g. autoantibodies, markers of systemic inflammation). Ideally AUCs and PPVs are obtained that are even higher than those observed here. Further research is needed to identify the best combination of biomarkers, and validate this in independent datasets. Preferably, this will be performed in cohorts that are even larger in size than those studied here, so that sufficient predictors can be included in the model without overfitting the data.

A strength of this study is that results were validated in an independent data-set. Since we used a data-driven approach to find predictors, validation was essential for confirmation of findings. PPVs of the third risk category (≥3 locations, no MCP-extensor peritendinitis) differed in the two cohorts, possibly due to small sample sizes in this subgroup. Reassuringly, the PPV was higher in the validation cohort. Further validation is needed to more reliably determine the PPV is this subgroup.

Part of the patients eligible for the validation cohort had subclinical inflammation and participated in a RCT and were therefore excluded. Although this exclusion of patients with a higher risk of arthritis development will decrease the overall probability of arthritis development, correcting for MRI-positivity ensures that within MRI-categories the predicted probabilities are still adequate (See Supplementary file 7).

Of note, 150 of the 225 patients in the discovery cohort were also included in a previously published analysis, which evaluated the association of a positive MRI with arthritis development [8]. The dataset at that time was insufficient to further evaluate separate inflammatory characteristics and to validate results.

A limitation is that in the first 77 of the 225 patients in the discovery cohort contrast enhanced and axial plane sequences were not performed in MTP-joints (Supplementary

file 4). Synovitis scoring without contrast is less specific [30]. Consequently the number of locations with subclinical inflammation could be slightly overestimated in part of the discovery cohort. However the PPVs of the number of locations were similar in the validation cohort, indicating that this effect seems limited.

Difference in follow-up duration between both cohorts could cause differences in effect sizes. Therefore, as all patients in both cohorts had ≥1 year follow-up, predictive values were determined at 1-year follow-up. This could have caused an underestimation of the conversion rates. More than 75% of patients in the discovery cohort converted to inflammatory arthritis <1-year, as can also be seen in Figure 2; indicating that somewhat higher PPVs can be expected when values would be determined after additional years of follow-up. This was indeed observed in the sensitivity analyses using 2-years of follow-up.

We used MRI to image subclinical joint inflammation. Although MRI is more sensitive than US, especially in the pre-arthritis phase [31], it is less feasible and more costly. This might currently hamper implementation of MRI in clinical practice in some centers or countries. Alternatively in other centers or regions, MRIs are already made to search for subclinical joint inflammation and the data presented here allow evidence-based use of the data provided by MRI.

In conclusion, tenosynovitis, particularly MCP-extensor peritendinitis is among the first tissues affected by RA. Incorporation of this feature and number of locations with subclinical inflammation improved prediction making for subgroups of patients, compared to MRI-positivity/MRI-negativity. These data allow evidence based use of MRI in patients presenting with CSA to predict RA development. Further research is now needed to combine the present MRI-data with other biomarkers to further improve risk stratification. Ultimately this may reduce the possible risk of overtreatment of patients at risk for RA.

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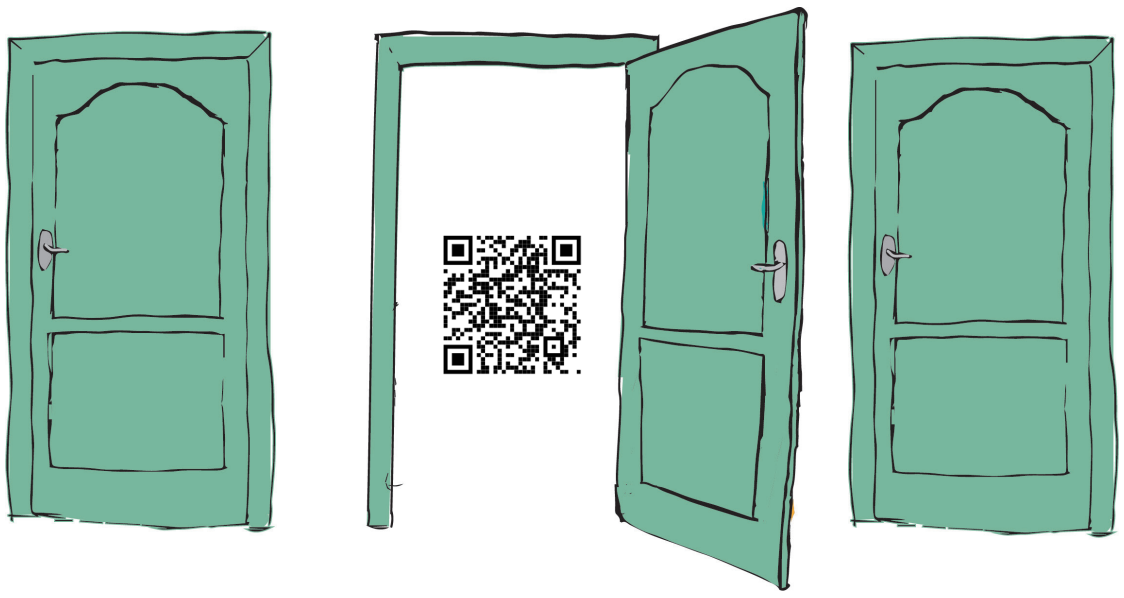
EARLY ARTHRITIS



CHAPTER 3

Tenosynovitis has a high sensitivity for early ACPA-positive and ACPA-negative RA: a large cross-sectional MRI study

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ABSTRACT

Objectives

Clinically evident tenosynovitis can be seen in established Rheumatoid arthritis (RA). Imaging research has recently shown that tenosynovitis at small joints occurs in early RA, contributes to typical RA symptoms (including joint swelling) and is infrequent in healthy controls. Imaging-detectable tenosynovitis is often not recognizable at joint examination, hence its prevalence can therefore be underestimated. We hypothesized that if MRI-detectable tenosynovitis is a true RA-feature, the sensitivity for RA is high, in both ACPA-positive and -negative RA, and lower in other diseases that are associated with enthesitis (such as Spondyloarthritis (SpA) and Psoriatic Arthritis (PsA)). So far, no large MRI-study addressed these questions.

Methods

Consecutive early arthritis patients (n=1211) from one health-care region underwent contrast-enhanced 1.5T MRI of hand and foot at diagnosis. MRIs were scored for synovitis and tenosynovitis by two readers blinded for clinical data. All included patients with ACPA-positive RA (n=250), ACPA-negative RA (n=282), PsA (n=88), peripheral SpA (n=24), reactive arthritis (n=30) and self-limiting undifferentiated arthritis (UA;n=76) were studied. Sensitivity was calculated.

Results

The sensitivity of tenosynovitis in RA was 85%; 88% for ACPA-positive RA and 82% for and ACPA-negative RA (p=0.19). The sensitivity for RA was significantly higher than for PsA (65%;p=0.001), SpA (53%;p<0.001), reactive arthritis (36%;p<0.001) and self-limiting UA (42%;p<0.001). The observed sensitivity of MRI-synovitis was 91% in RA and ranged 83-54% in the other groups.

Conclusions

MRI-detected tenosynovitis has a high sensitivity for early ACPA-positive and ACPA-negative RA. This supports both juxta-articular (tenosynovitis) and intra-articular synovial involvement is characteristic for RA.

KEY MESSAGES

What is already known about this subject?

- Imaging research has identified tenosynovitis at small joints in early RA and its contribution to typical RA symptoms (including joint swelling).
- So far, no large MRI-study in consecutive patients determined the sensitivity of imaging detected tenosynovitis. We hypothesized that if tenosynovitis at small joints is a true RA-feature, the sensitivity for RA is high, in both ACPA-positive and -negative RA, and lower in diseases that are associated with enthesitis (e.g. SpA, PsA).

What does this study add?

- This is the first study demonstrating the sensitivity of tenosynovitis in RA, which is high (>80%), not different for ACPA-positive and ACPA-negative RA, and lower in spondyloarthropathies.
- The sensitivity of tenosynovitis in wrist, MCP and MTP joints was comparable to synovitis, a well-established RA-feature. This supports that both juxta-articular (tenosynovitis) and intra-articular synovial involvement is characteristic for RA.

How might this impact on clinical practice or future developments?

- This may fuel future research into the role of juxta-articular synovial inflammation in the pathogenesis of RA.

INTRODUCTION

Clinically evident tenosynovitis can be seen in established Rheumatoid arthritis (RA), for example at the back of the hand in patients with longstanding disease.[1] Clinically evident tenosynovitis during the disease course is less frequent than joint swelling, which is generally interpreted as a sign of synovitis. However, in contrast with clinically evident tenosynovitis, imaging studies using advanced high resolution imaging (MRI, US) have recently shown the presence of tenosynovitis in small joints of hands and feet. Imaging-detected tenosynovitis has been shown to occur in early RA and pre-RA phases, additionally it is also noted to be a strong predictor for RA development in undifferentiated arthritis and arthralgia.[2] Conversely, MRI-detected tenosynovitis is infrequent in healthy controls.[2] Furthermore, imaging-detected tenosynovitis is believed to underlie typical RA-symptoms of pain, functional limitations and morning stiffness, and it can contribute to joint swelling.[3] MRI-detectable tenosynovitis is often not recognizable at joint examination [3] and accurate detection requires high resolution contrast-enhanced MRI.[4-6] Therefore its prevalence may have been thus far underestimated. Ultrasonography is used more often than MRI but has a lower sensitivity for tenosynovitis compared to high-resolution contrast-enhanced MRI.[4-7] Consequently, the sensitivity of tenosynovitis at the level of small joints for RA remains unknown.

Previous studies that reported on the prevalence of MRI-detected tenosynovitis in RA studied selected sets of patients; only one study included a representative consecutive sample, allowing to determine the sensitivity of tenosynovitis for RA (see literature overview in Supplementary 1).[8] To our knowledge, no studies have evaluated the prevalence of tenosynovitis in ACPA-positive and ACPA-negative RA, nor has it been studied in MTP joints, a preferential location for RA. Furthermore, only three small studies compared the prevalence of MRI-detected tenosynovitis to other diagnoses that are known to be associated with enthesitis, such as spondyloarthropathies.[9-11] Enthesitis and tenosynovitis are sometimes mixed up; enthesis is inflammation of the insertion of the tendon to the bone whereas tenosynovitis is inflammation of the synovial sheath that surrounds many tendons in the hands and forefeet.

The increasing amount of data on the value of tenosynovitis in RA prompted us to conduct this study. We hypothesized that if MRI-detectable tenosynovitis is a true RA-feature, the sensitivity for RA is high, and is similar for anti-citrullinated protein antibodies (ACPA)-positive and ACPA-negative RA. This hypothesis was based on the clinical presentation of ACPA-positive and ACPA-negative RA being similar, despite the differences in risk factors and outcome.[12] Furthermore, if tenosynovitis is a true RA-feature, the sensitivity of tenosynovitis for other diseases should be low. The spondyloarthropathy group (including psoriatic arthritis (PsA), peripheral spondylarthritis (SpA) and reactive arthritis) are important to compare due to the role of enthesitis and dactylitis in these diseases. Also, self-limiting undifferentiated arthritis (self-limiting UA) is interesting, as these patients ultimately do not develop chronic arthritis.

To address these questions, we took advantage of the Leiden early arthritis cohort (EAC), a large representative consecutive cohort of >1200 early arthritis patients who received a contrast-enhanced, 1.5T extremity MRI of wrist, MCP and MTP-joints at presentation to the rheumatology outpatient clinic. Patients with RA, PsA, peripheral SpA, reactive arthritis and self-limiting UA were studied for the prevalence of tenosynovitis, and also its relation to synovitis.

METHODS

Patients

Since 1993, consecutive early arthritis patients (<2 years symptom duration) presenting to the rheumatology outpatient clinic, were included in the Leiden EAC. This is the only rheumatology referral center in this region. Therefore a representative sample of early arthritis patients presents itself at this outpatient clinic. Also, a short waiting list and a special early arthritis recognition clinic without a waiting list, opened in 2010, safeguards high accessibility, this is reflected in a short symptom duration at inclusion in the EAC. More information on this inception cohort is available elsewhere.[13] Briefly, patient-characteristics, disease activity and laboratory parameters were obtained at baseline, 4-months, 12-months and yearly thereafter. From August-2010 onwards MRIs were performed at baseline.

The definite diagnosis was determined after 1 year based on routinely available data (MRI-data were not reported to clinicians). RA was defined as clinical diagnosis plus fulfillment of the 1987- or 2010-classification criteria. PsA, SpA and reactive arthritis diagnoses were made by the rheumatologist based on clinical presentation and not based on classification criteria as these are inappropriate for diagnosis of individual patients in daily practice. PsA patients had psoriasis of the skin and poly-arthritis and were treated for PsA. Peripheral SpA patients had axial spondylarthritis and arthritis of one or more peripheral joints and were treated for peripheral SpA. Self-limiting UA patients were diagnosed with UA by the rheumatologist but had resolving joint swelling and complaints within 1-year without DMARD treatment (systemic DMARDs, biological DMARDs or (intra-articular) glucocorticoids), resulting in a subsequent release from care. All patients were consecutively included and no selection was made on clinical characteristics.

Patients included in the EAC between August-2010 and March-2020 were evaluated in the present study (Flowchart in Supplementary 2). A minority of patients did not undergo an MRI evaluation (mostly due to logistical reasons such as MRI-maintenance) and some MRIs were of insufficient quality (e.g. no contrast-enhanced sequences or insufficient fat suppression), implying missingness completely at random. Baseline characteristics were similar in patients with and without MRI, substantiating this assumption (Supplementary 3). Consequently, we studied a representative consecutive sample of 1211 early arthritis patients that received an MRI at baseline.

MRI

MRI was performed at baseline (before DMARD-initiation). Wrist, metacarpophalangeal (MCP(2-5)), and metatarsophalangeal (MTP(1-5))-joints on the most painful side at baseline (dominant side in case of symmetric symptoms) were imaged with 1.5TMRI (GE,Wisconsin,USA). Contrast-enhanced T1-weighted FSE fatsat sequences of the wrist and MCP sequences were obtained in all patients. In June-2013, instead of axial T1 and T2-weighted FSE fatsat sequences in the axial plane, contrast-enhanced T1-weighted FSE fatsat sequences in both the coronal and axial plane of the MTPs were added to the protocol. This allowed for assessment of the influence of the MTPs on the sensitivity of tenosynovitis in 823 patients. Supplementary 4 provides a detailed scan and scoring-protocol.

MRIs were scored for synovitis and tenosynovitis in line with RAMRIS and the method of Haavardsholm by two experienced readers, blinded to any clinical data (Supplementary 4).[14-16] Intraclass correlation coefficients were excellent (≥0.93; Supplementary 5). Tenosynovitis and synovitis were considered present when both readers considered the feature present at the same location. This stringent definition was chosen to minimize false-positive results.

Statistical analysis

The sensitivity of tenosynovitis in early RA was calculated using all described joints in both hand and foot. This was repeated stratified for ACPA-status and compared to other diagnoses. To assess whether high tenosynovitis scores were more prevalent in RA, continuous scores for different diagnoses were plotted in a violin plot and tested with Mann-Whitney tests. For comparison, the sensitivity of MRI-detected synovitis was calculated.

To study the influence of the stringent cut-off chosen, analyses were repeated in RA patients with a less stringent cut-off: a feature was considered present when one of both readers scored the feature at that location.

In another sub analysis we evaluated the contribution of tenosynovitis at the MTP level to the sensitivity of tenosynovitis for RA, by repeating the analyses while excluding the MTPs. Although the feet are a preferential location for RA, previous studies did not scan the MTPs and omitting the MTPs increases comparability to previous studies on the prevalence of tenosynovitis. In addition it allows for inclusion of patients in which MTP tenosynovitis could not be properly scored due to MRI-protocol differences (n=388).

To investigate the distribution of synovitis and tenosynovitis, the prevalence of tenosynovitis and synovitis was assessed at the joint level in RA and other diagnoses. Moreover, simultaneous presence of those features was assessed for the individual MTPs and MCPs and the wrist. To avoid multiple testing, no statistics were performed on these joint level analyses.

Fisher's exact test was used. A narrative literature review on the prevalence of tenosynovitis in RA was performed (Supplementary 1). Results are reported according the Standards for Reporting of Diagnostic Accuracy Studies (STARD)-guidelines (Supplementary 6). No formal pre-specified study protocol was submitted prior to analyses. Patient partners were involved in design of the EAC-cohort. R4.0.0 was used. Two-sided p-values <0.05 were considered significant.

RESULTS

1211 patients included in the EAC received MRIs: 532 had RA (n=250 ACPA-positive; n=282 ACPA-negative), 88 PsA, 24 peripheral SpA, 30 reactive arthritis and 76 self-limiting UA. Baseline characteristics are shown in Table 1 and swollen joint distribution in Supplementary 7. The diagnoses of the 461 patients that were not studied are shown in Supplementary 8.

Table 1: Baseline characteristics of early arthritis patients presenting with rheumatoid arthritis, psoriatic arthritis, peripheral spondyloarthritis, reactive arthritis and self-limiting UA

	Rheumatoid arthritis (n=532)	Psoriatic arthritis (n=88)	Peripheral spondyloarthritis (n=24)	Reactive Arthritis (n=30)	Self-limiting undifferentiated arthritis (n=76)
Women, n (%)	343 (65)	38 (43)	11 (46)	18 (60)	41 (54)
Age in years, mean (SD)	59 (14)	49 (15)	38 (14)	47 (15)	50 (15)
Symptom duration, weeks median (IQR)	12 (6-29)	16 (7-47)	13 (5-39)	4 (2-7)	8 (3-16)
ACPA, n (%)	250 (47)	2 (2)	1 (4)	0 (0)	0 (0)
66-SJC, median (IQR)	5 (2-10)	2 (1-4)	2 (1-4)	2 (1-4)	1 (1-2)

Table 1: Continued.

	Rheumatoid arthritis (n=532)	Psoriatic arthritis (n=88)	Peripheral spondyloarthritis (n=24)	Reactive Arthritis (n=30)	Self-limiting undifferentiated arthritis (n=76)
68-TJC, median (IQR)	5 (3-7)	3 (1-5)	2 (1-3)	2 (1-5)	1 (1-2)
ESR, median (IQR)	28 (11-41)	17 (6-33)	33 (9-58)	21 (9-33)	11 (6-22)
VAS general health, median (IQR)	70 (50-80)	68 (50-80)	70 (60-80)	70 (40-80)	60 (40-70)
HAQ, median (IQR)	0.9 (0.5-1.5)	0.5 (0.1-0.9)	0.4 (0.2-0.8)	0.8 (0.3-1.0)	0.4 (0.0-0.9)

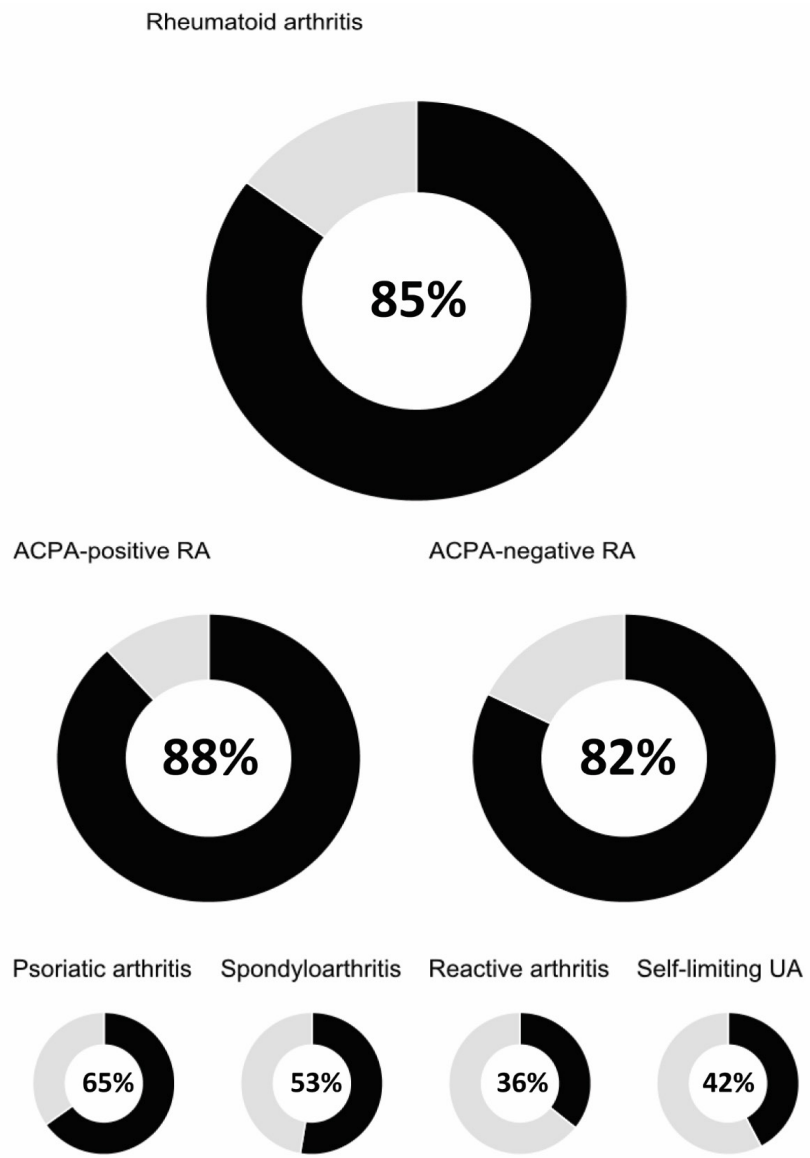
Legend: n, number of patients; SD, standard deviation; IQR, inter quartile range; ACPA: anti-citrullinated protein antibodies; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; VAS, visual analogue scale; HAQ, health assessment questionnaire; UA, undifferentiated arthritis

In early RA, the sensitivity of imaging-detected tenosynovitis in the hand and foot joints in early RA was 85%. Sensitivity was 88% in ACPA-positive and 82% ACPA-negative RA (p=0.19; Figure 1). This was 65% in PsA (p<0.001 vs RA), 53% in peripheral SpA (p=0.001), 36% in reactive arthritis (p<0.001) and 42% in self-limiting UA (p<0.001). Analyses of continuous scores revealed that higher tenosynovitis scores were only prevalent in RA (Figure 2; all p<0.001).

The sensitivity of tenosynovitis was compared to MRI-detected synovitis in the hand and foot joints, an established feature of RA. The sensitivity of MRI-detected synovitis in wrist, MCP and MTP joints in RA was 91% (Figure 3) and 91% in ACPA-positive RA and 90% in ACPA-negative RA. This was 85% in PsA (p=0.08 vs RA), 58% in peripheral SpA (p<0.001), 47% in reactive arthritis (p=0.002) and 54% in self-limiting UA (p<0.001). Considering both features together, 94% of RA patients had synovitis or tenosynovitis in wrist, MCP or MTP joints. If a less stringent definition was considered (feature at least observed by one reader at that location), only 6 patients had no (teno-)synovitis in the joint regions that were scanned, mostly due to receiving MRIs at the least affected side, thereby diverging from protocol

Analyses were repeated excluding the MTP joints to ascertain the impact of tenosynovitis in the feet. Similar results were obtained for tenosynovitis: Sensitivity in early RA was 79% (Supplementary 9), 81% in ACPA-positive and 78% in ACPA-negative RA (p=0.34). In PsA, peripheral SpA, reactive arthritis and self-limiting UA, sensitivity was 56%, 24%, 36% and 42%, respectively (all p<0.001). For synovitis, the sensitivity in early RA decreased from 91% to 82% upon omitting the feet (Supplementary 10).

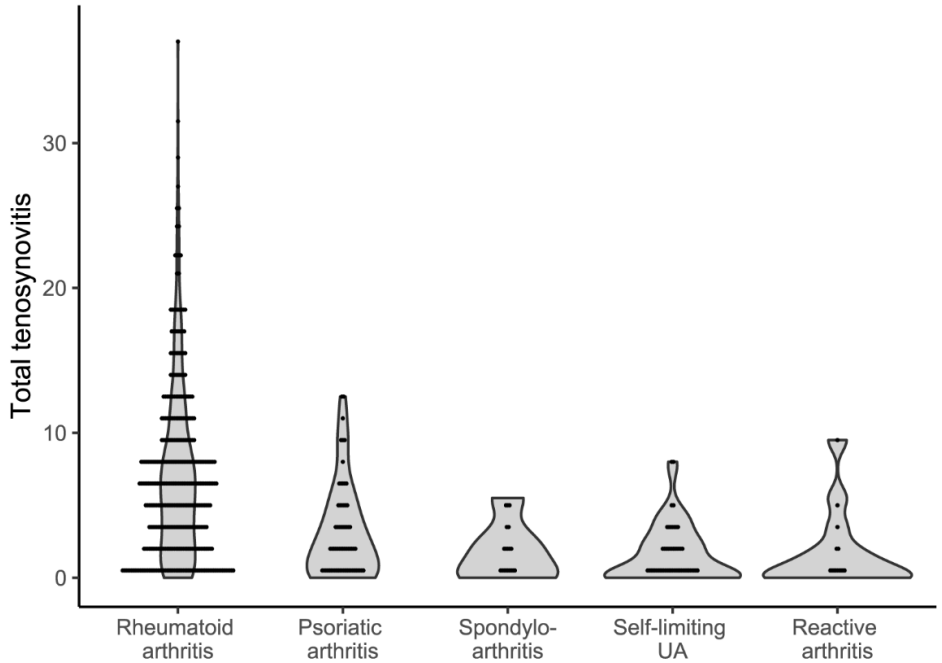
Figure 1: Presence of tenosynovitis (in black) in wrist, MCPs and MTPs, in rheumatoid arthritis, stratified for ACPA-status and compared to other diseases



Legend: RA: Rheumatoid arthritis; ACPA: anti-citrullinated protein antibodies; UA: undifferentiated arthritis

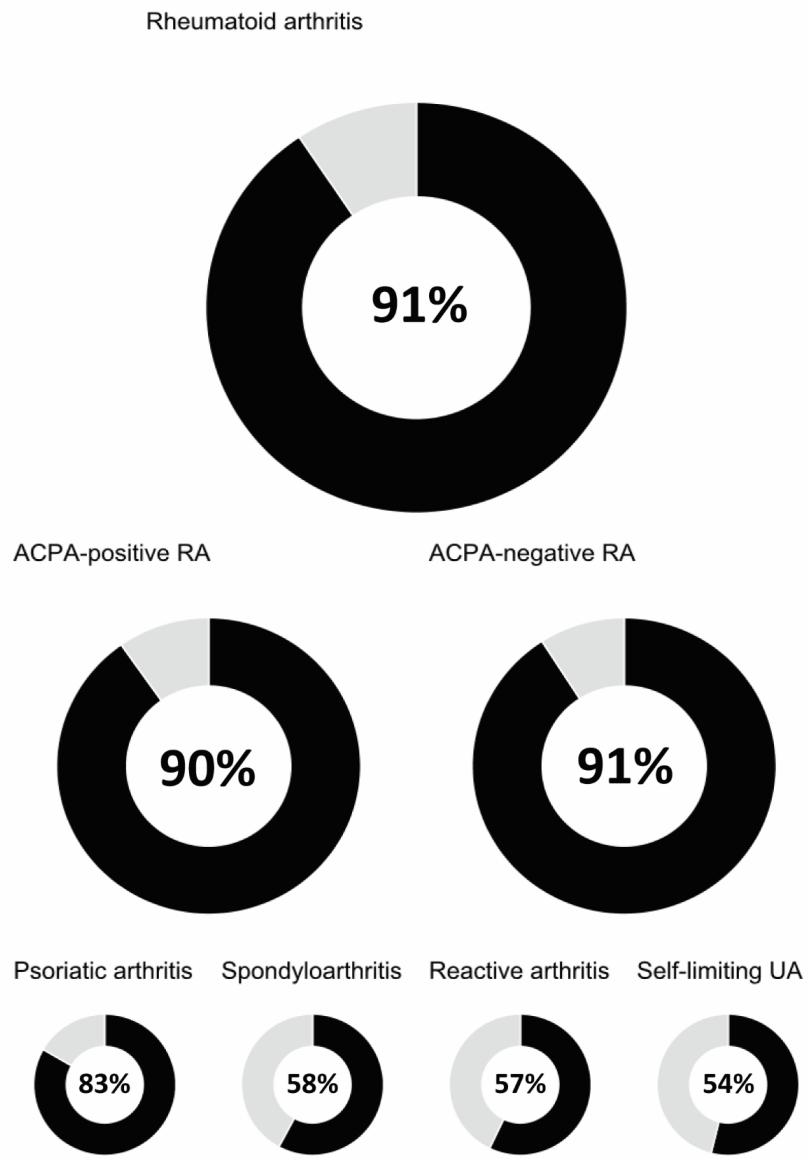
Prevalence of tenosynovitis and synovitis were also assessed at the joint level. This revealed a numerically higher prevalence of tenosynovitis at the level of the individual MCP and wrist joints in RA than in other arthritides. For the MTPs, the differences observed were unclear (Figure 4-6). Information on the flexor and extensor sides of the MCP and MTP joints is provided in Supplementary 11-12; showing similar distributions. Finally, the simultaneous presence of synovitis and tenosynovitis was assessed in RA patients on joint level. As presented in Supplementary file 13 synovitis and tenosynovitis were most often simultaneously present in the same joint.

Figure 2: Total tenosynovitis scores in RA and similar diagnoses, showing that all high tenosynovitis scores are found in RA patients



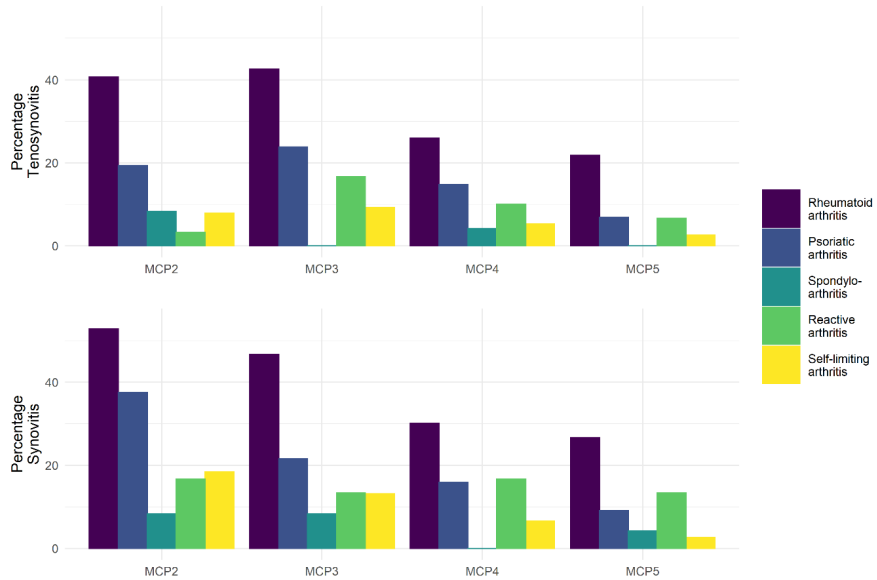
Legend: Horizontal line width represents number of patients with that mean of tenosynovitis scores of two readers, rounded to a whole number. Light grey violins represent the density of patients with that mean tenosynovitis scale, scaled such that all violins have the same area, irrespective of the number of patients in that group.

Figure 3: Presence of synovitis (in black) in wrist, MCPs and MTPs, in rheumatoid arthritis, stratified for ACPA-status and compared to other diseases



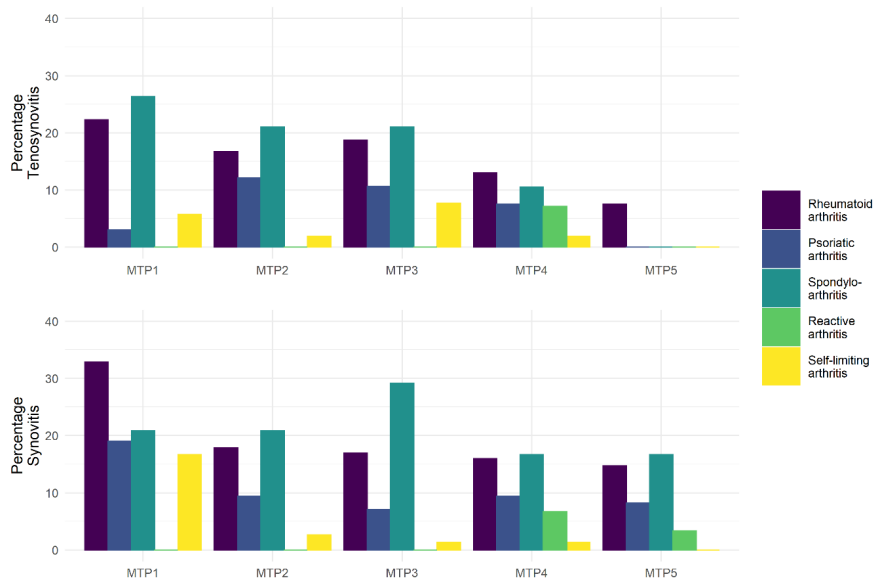
Legend: RA: Rheumatoid arthritis; ACPA: anti-citrullinated protein antibodies; UA: undifferentiated arthtritis

Figure 4: Presence of tenosynovitis and synovitis in the individual metacarpophalangeal (MCP) joints in RA and other arthritides showing a higher prevalence in RA



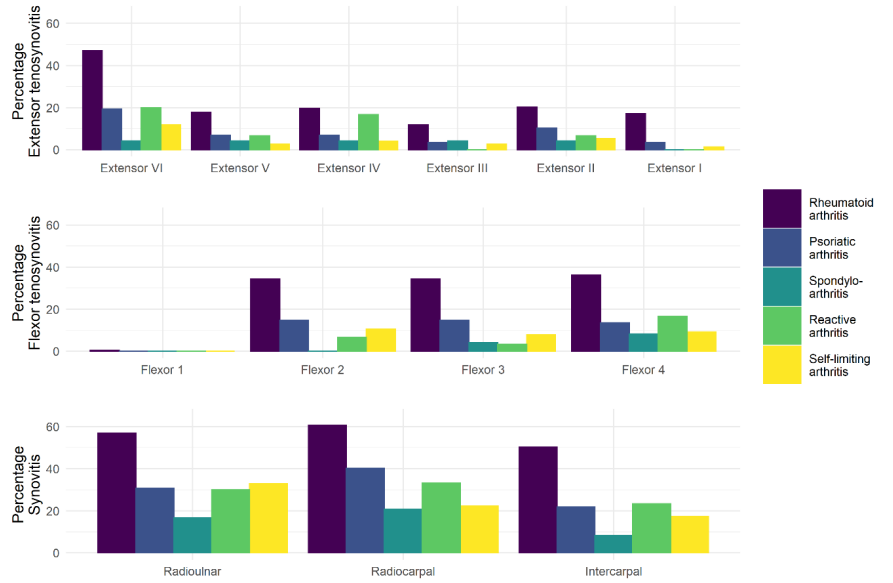
Legend: Features were considered present if both readers considered the feature present at the same location. MCP: metacarpophalangeal

Figure 5: Presence of tenosynovitis and synovitis in the individual metacarpophalangeal (MTP) joints in RA and other arthritides



Legend: Features were considered present if both readers considered the feature present at the same location. MTP: metatarsophalangeal

Figure 6: Presence of tenosynovitis and synovitis in the individual locations within the wrist in RA and other arthritides showing a higher prevalence in RA. Because wrist tendon sheaths cannot be mapped to synovitis locations, the prevalence of synovitis and tenosynovitis cannot be compared at individual locations



Legend: Features were considered present if both readers considered the feature present at the same location.

DISCUSSION

We performed a large MRI study on consecutively included early arthritis patients over 10 years, all receiving an MRI scan at baseline. Selecting RA patients from this consecutive sample enabled us to determine the sensitivity of MRI-detectable tenosynovitis in early RA. This is the first study to demonstrate the sensitivity of tenosynovitis in RA is high (>80%), does not differ between ACPA-positive and ACPA-negative disease and is lower in spondylarthritis diseases. The sensitivity of tenosynovitis was comparable to synovitis, a well-established RA-feature. This further confirms that RA is both a juxta-articular (tenosynovitis) and intra-articular (synovitis) disease.

Sensitivity of tenosynovitis was comparable to that of synovitis for RA (85% and 91% respectively). Also, 94% of RA patients had synovitis and/or tenosynovitis in wrist, MCP or MTP joints. In RA-patients tenosynovitis and synovitis predominantly presented in the same joints (Supplementary 13), implying that local inflammation manifests both juxta- and intra- articularly. Whilst synovitis frequency in RA was comparable to that of other diagnoses assessed (e.g. 83% in PsA), tenosynovitis was more frequent in RA than

other diagnoses. Therefore it can be concluded that the characteristics of tenosynovitis in RA are similar to synovitis, a well-known RA-feature, but that tenosynovitis is less frequently observed in other diagnoses.

Although ACPA-positive and ACPA-negative RA have different risk factors, outcomes and are hypothesized to have a different pathogenesis, the clinical presentation of both ACPA-subsets of RA is similar.[12] Our data shows that tenosynovitis is also highly frequent in both disease subsets. This underlines the notion that, although the pathogenesis and the severity of the disease course are different between ACPA-positive and ACPA-negative RA, the disease presentation is similar at the time of diagnosis, both with respect to the clinical presentation and high resolution imaging features.

ACPA-negative RA encompasses a heterogenous set of patients, which can raise concerns about phenotypic misclassification. Our data addressed this issue by confirming there is a difference in tenosynovitis between ACPA-negative RA and other inflammatory arthritides, such as PsA. This is in line with findings from recent studies on metabolites.[17] Together this supports the idea that ACPA-negative RA is a separate entity and not only a selection of patients that have other forms of arthritis that are misclassified.

We studied self-limiting arthritis in the form of reactive arthritis and self-limiting UA, the clinical distinction between the two being a recognized infectious illness preceding the onset of arthritis. Remarkably, both groups had a similar prevalence of both synovitis and tenosynovitis, possibly suggesting an overlapping or similar underlying disease mechanism between the two conditions.

Dactylitis is a known feature of PsA; the classic 'sausage digits' are caused by synovitis, soft tissue edema and tenosynovitis.[18,19] Importantly, this affects the digits mostly distal from the MCP- and MTP-joints. These distal areas were not imaged. Hence the current findings on tenosynovitis and its frequent occurrence in RA concern tenosynovitis at the level of wrist, MCP- and MTP-joints.

The feet are recognized as a preferential location for RA, however no previous studies have investigated the prevalence of the tenosynovitis in this location. The sensitivity of MRI-detected tenosynovitis in RA, including the feet (85%), was comparable to the sensitivity without the feet (79%). Previous studies that did not include the feet reported prevalences ranging from 43%-84% but most were ≥75% (Supplementary 1). The relatively small difference between the prevalence of tenosynovitis when the feet are included or excluded suggest that patients that have tenosynovitis at MTP level often also have tenosynovitis in the hand joints.

To our knowledge, this is the first large study on the sensitivity of MRI-detected tenosynovitis in RA to make a direct comparison to other inflammatory rheumatological diseases that are associated with enthesitis. The setup of the EAC cohort ascertains a representative sample of early arthritis patients are included. Therefore we were able calculate an estimate of the sensitivity of imaging-detected tenosynovitis in the general RA population, as opposed to describing a prevalence in a selection of RA-patients as typically done in previous research.

A limitation of this study is MTPs of some patients could not be scored for tenosynovitis due to a different MRI protocol. Reassuringly, a large number of patients (833 of which 362 were diagnosed with RA) remained, in whom the sensitivity of tenosynovitis and synovitis could be calculated while including the MTPs.

The current study aimed to increase the understanding of the frequency of tendon sheath involvement in RA. Nonetheless the MRI-protocol that we used might not be feasible in clinical practice: barriers include high cost and availability of MRI. Unfortunately, some clinical manifestations related to imaging-detectable tenosynovitis in clinical practice, such as swollen joints, incomplete fist closure, and also ultrasound-detectable tenosynovitis, have low sensitivity for imaging-detectable tenosynovitis.[3,7,20] Also, low-field MRI or MRI without contrast-enhancement is less sensitive for detection of tenosynovitis.[4-6] Therefore high field (≥1.5 Tesla) MRI is the ideal test for understanding and depicting which tissues are involved in RA.

Given the high sensitivity of tenosynovitis for RA and the lower prevalence in other inflammatory arthritides, future research could help to elucidate in which patients and phase of disease it is cost-effective to perform an MRI to detect tenosynovitis and distinguish RA from other diseases in an early disease stage. Moreover, it is relevant to study the morphologic, histologic and molecular characteristics of tenosynovitis in early RA. These are still unexplored areas that warrant further investigations.

In conclusion, this is the first large consecutive study on MRI-detected tenosynovitis in early arthritis patients and we have demonstrated that the large majority of RA patients have tenosynovitis at the level of small hand and foot joints, irrespective of ACPA-status. This further confirms that tenosynovitis, aside from synovitis, is a true RA feature and may fuel future research into the role of juxta-articular synovial inflammation in the pathogenesis of RA. Finally this study provided an example that a large MRI study can expand the knowledge on novel characteristics of RA, even 70 years after the first description of RA as a separate disease entity.[21]

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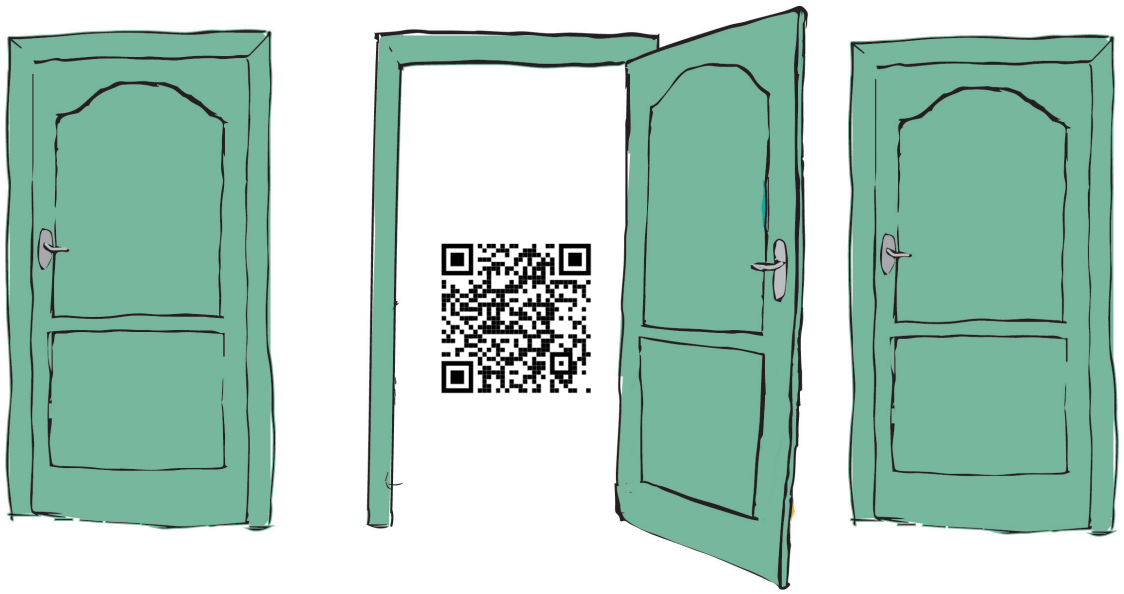

CHAPTER 4

The increasing incidence of autoantibody negative RA is replicated and is partly explained by an aging population

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With great interest we read the recently published report by Myasoedova et al. in which a significant increase in incidence of rheumatoid factor (RF)-negative rheumatoid arthritis (RA) was found, in contrast to RF-positive RA.[1] Studies on trends of RA-incidence stratified for autoantibodies are scarce. Moreover, both an increase and decrease in incidence of RF-negative RA has been reported.[2,3] Because validation is important, we determined trends in incidence of RA over two decades in our region.

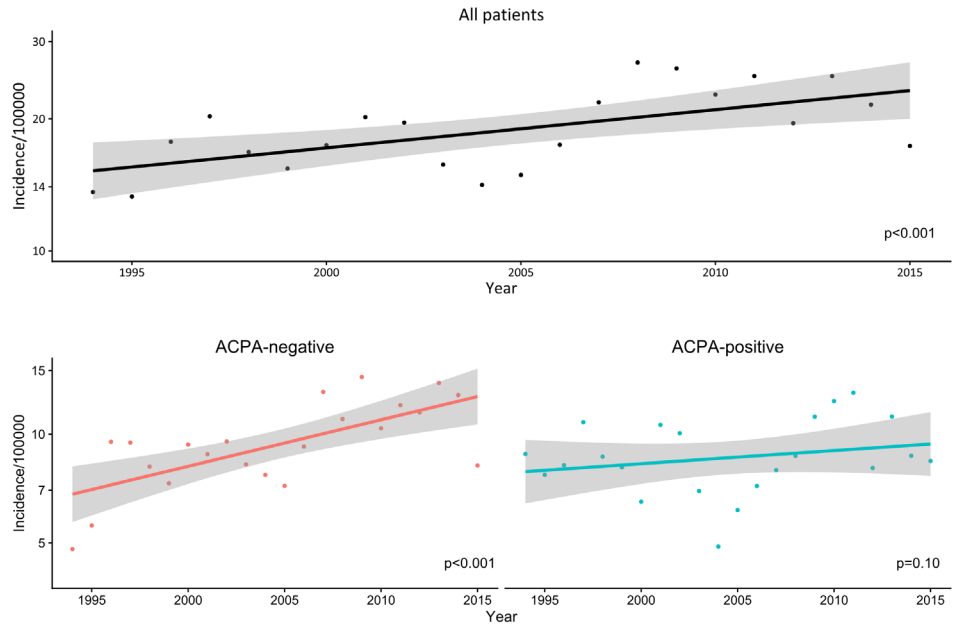
We defined autoantibody-positivity as auto-citrullinated protein antibodies (ACPA)-positivity, since RF is less specific for RA and more often present in healthy controls, especially at older age.[4] Second, because autoantibody-negative RA has an higher age-of-onset than autoantibody positive RA,[5] we hypothesized that part of the incidence increase is explained by aging of the population. Therefore, we also assessed the influence of the population age-distribution on the trends of incidence of RA.

Incidence rates were calculated based on the inclusion rate of RA patients in the Leiden Early Arthritis Cohort (EAC). The Leiden University Medical Center (LUMC) is the only rheumatology referral center within the Leiden area and inclusion in the EAC of newly presenting early arthritis patients has been part of regular care since 1993.[6] All consecutively included RA-patients (defined as clinical diagnosis plus fulfilling the 1987 or 2010-criteria within 1 year) included in the EAC between 1994-2015 were studied.

First, we calculated crude incidence rates per year using the number of incident cases as the numerator and total population counts from the NUTS-3 (Nomenclature of Territorial Units for Statistics) region around Leiden as the denominator.[7] Trends over time were analyzed with Poisson regression. Next, to assess the influence of age-changes in the Leiden population, a three degree of freedom spline of age was included in the Poisson models. All analyses were stratified for ACPA (anti-CCP2)-status; which was determined after inclusion but rarely by GPs in line with Dutch guidelines.[8]

1697 RA-patients were included between 1994-2015 (mean age 57, 66% female, 48% ACPA-positive). For the total RA population, a crude incidence increase was observed (β=0.020 (95% confidence interval 0.012;0.027); Figure 1). This estimate approximates the proportion increase per year, where 0.02 translates to ~2% increase per year. Stratification for ACPA-status revealed that the crude incidence of ACPA-negative RA increased (0.028 (0.017;0.039)) while ACPA-positive RA did not significantly increase (0.009 (-0.002;0.021)). We thereby replicated the findings of Myasoedova et al. Further stratification for IgM-RF-status within ACPA-negative RA revealed no significant differences in the increase in crude incidence between RF-positive ACPA-negative and RF-negative ACPA-negative RA (0.039 (0.017;0.061) versus 0.023 (0.011;0.036); p=0.22)).

Figure 1: Crude incidence of RA in the Leiden area 1994-2015 in all patients (above) and stratified for ACPA (below)

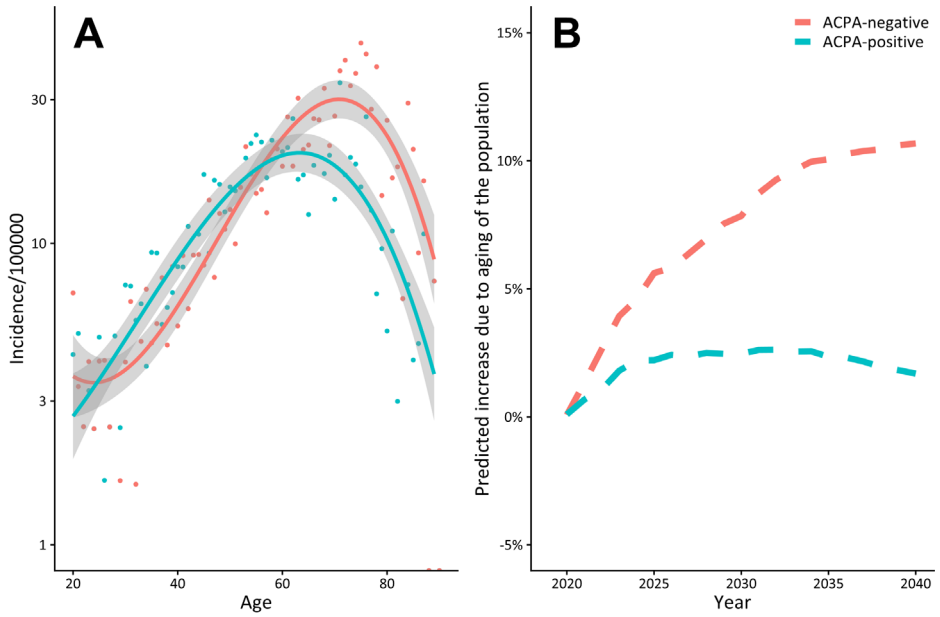


Legend: Y-axis are presented on the log-scale. Dots depict the observations per year. Fitted linear lines are depicted in bold and confidence intervals in light grey.

ACPA-negative RA had the peak incidence at higher age (mean age at diagnosis 59 vs 54; p<0.001; Figure 2A), which is in line with previous observations.[5] We then adjusted incidence rates for the changes in age distribution in our health care region 1994-2015. This revealed lower estimates in both ACPA-subsets, suggesting that part of the crude incidence increase was due to aging. After this age-correction, the incidence of ACPA-negative RA still showed some remaining increase over time (0.017 (0.006;0.028)). Also here there was no increased incidence in ACPA-positive RA (0.000 (-0.011;0.012)).

Because we observed that the increase in incidence of the past decades was partly explained by aging of the population, and it is known that the population will age even more, we estimated the further increase in ACPA-negative RA for the coming two decades based on ageing using age-specific Dutch population prognoses of Statistics Netherlands.[9] As presented in Figure 2B, the estimated increase of new RA cases the next twenty years due to aging of the population is 11% in ACPA-negative RA and 2% in ACPA-positive RA.

Figure 2: Crude incidence per age (A), and predicted increase in incidence due to aging of the Dutch population (B), both for ACPA-negative and ACPA-positive RA



Legend (A): Y-axis are presented on the log-scale. Dots depict the observations per age. Fitted lines are depicted in bold and confidence intervals in light grey.

Our analyses are based on the assumption that all incident RA cases in the region are included in the EAC. This assumption is supported by the fact that the LUMC is the only referral center in the region. Importantly, the referral region and strategy has not changed during the last two decennia, hence if a proportion of novel RA-patients is not included in the cohort, this is presumably similar over time and does not affect our results on trends over time.

In conclusion, we found an increasing incidence of ACPA-negative RA that was absent in ACPA-positive RA, which is line with the findings of Myasoedova et al. Moreover, we showed that the increase in ACPA-negative RA was in part explained by aging of the population. This will make ACPA-negative RA more prevalent the coming years and promotes the need for research in this subset of RA.

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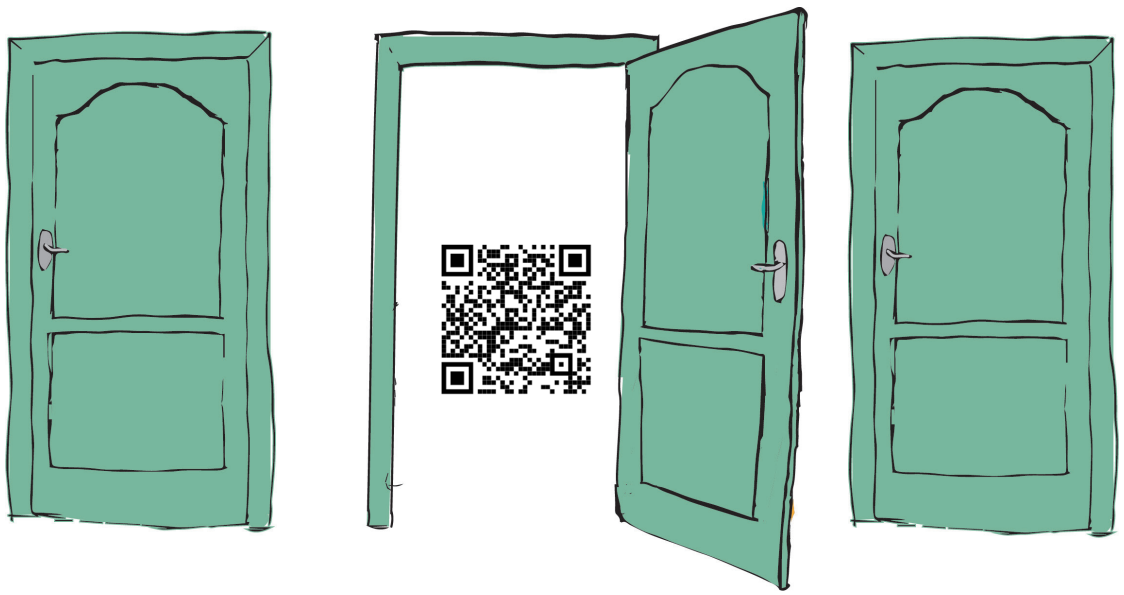

CHAPTER 5

The value of imaging detected joint inflammation in explaining fatigue in RA at diagnosis and during the disease course - a large MRI study

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ABSTRACT

Objective

Fatigue in rheumatoid arthritis (RA) is hypothesized to be caused by inflammation. Still ~50% of the variance of fatigue in RA cannot be explained by the disease activity score (DAS), nor by background or psychological factors. Since MRI can detect joint inflammation more sensitively than the clinical joint counts as incorporated in the DAS, we hypothesized that inflammation detected by MRI could aid in explaining fatigue in RA at diagnosis and during follow-up.

Methods

526 consecutive RA-patients were followed longitudinally. Fatigue was assessed yearly on a numerical rating scale. Hand and foot MRIs were performed at inclusion, after 12 and 24-months in 199 patients and were scored for inflammation (synovitis, tenosynovitis and osteitis combined). We studied whether RA-patients with more MRI-inflammation were more fatigued at diagnosis (linear regression), whether the 2-year course of MRI-inflammation associated with the course of fatigue (linear mixed models) and whether decrease in MRI-inflammation in year-1 associated with subsequent improvement in fatigue in year-2 (cross-lagged models). Similar analyses were done with DAS as inflammation measure.

Results

At diagnosis, higher DAS-scores were associated with more severe fatigue (p<0.001). However, patients with more MRI-inflammation were not more fatigued (p=0.94). During 2-year follow-up, DAS decrease associated with improvement in fatigue (p<0.001), but MRI-inflammation decrease did not (p=0.96). DAS decrease in year-1 associated with fatigue improvement in year-2 (p=0.012), as did MRI-inflammation decrease (p=0.039), with similar effect strength.

Conclusion

Sensitive measurements of joint inflammation did not explain fatigue in RA at diagnosis and follow-up. This supports the concept that fatigue in RA is partly uncoupled from inflammation.

KEY MESSAGES

What is already known about this subject?

- Fatigue in rheumatoid arthritis (RA) contributes majorly to the disease burden and is hypothesized to be caused by inflammation. However, ~50% of the variance of fatigue in RA cannot be explained by clinical measures of joint inflammation, and neither by other factors such as psychological factors or pain.
- Since MRI can detect joint inflammation more sensitively than the clinical joint counts as incorporated in the DAS, we hypothesized that joint inflammation of the hands and feet as detected by MRI could aid in explaining fatigue in RA

What does this study add?

- The association of fatigue and MRI-inflammation at baseline and during the disease course was studied in a consecutive cohort of >500 RA patients. In this large cohort, we observed that MRI-inflammation did not explain fatigue, both at baseline and during the disease course.

How might this impact on clinical practice or future developments?

- This suggests there is a ceiling effect for explaining fatigue by inflammation and supports the concept that fatigue in patients with classified RA is in part disconnected from inflammation.
- Consequently, this implies that aiming at imaging remission does not lower fatigue in RA

INTRODUCTION

Fatigue in rheumatoid arthritis (RA)-patients contributes majorly to the disease burden.[1] Despite the fact that treatment strategies have improved dramatically during the last decades, persistent fatigue is still a major issue and present in up to 80% of RA-patients.[2] Because of this, patient partners promote awareness of fatigue and stimulate research into fatigue, as an increased understanding of the underlying process may lead to novel ways to address fatigue in RA.[1]

Studies examining fatigue at diagnosis showed that it is associated with inflammation, expressed by the disease activity score (DAS) and by background factors such as young age, female sex, low education level, smoking, and mental health.[2,3] Studies on fatigue during the disease course have also shown that fatigue is associated with DAS, background factors and pain.[2,4,5] In addition, it has been shown that early intensive treatment and consequent early remission are associated with subsequent fatigue improvement.[6,7] However, only ~40% of the variance of fatigue in RA is explained by clinical inflammation.[4,8] Even when also considering other (possible) explanatory factors, such as mental health, disability and pain, ~50% of the variance of fatigue in RA remains unexplained.[4,5]

Imaging detects local joint inflammation more sensitively than the joint counts as incorporated in the DAS. Therefore we hypothesized that MRI-detected joint inflammation could aid in understanding fatigue in RA. To our knowledge, no studies have been performed so far to determine if part of fatigue is associated with imaging detected joint inflammation in the hands and feet, neither at the time of diagnosis, or during the course of the disease. Therefore, while fatigue causes a great burden of disease and is considered to be a consequence of inflammation, the contribution of imaging detected joint inflammation to fatigue in RA is undetermined; This prompted the current study. Our aim was to determine the contribution of MRI-inflammation to fatigue at diagnosis and during the disease course. We addressed three questions: 1) Are RA-patients with more MRI-inflammation at diagnosis more fatigued? 2) Is MRI-inflammation during the disease course associated with the course of fatigue? and 3) Is decrease in MRI-inflammation followed by decreasing fatigue? To confirm previous research on the association of DAS and fatigue and to allow for comparison of different forms of inflammation, these three analyses were repeated with DAS as an inflammatory measure.

METHODS

Patients

The Leiden early arthritis clinic (EAC) includes consecutive early arthritis patients (<2 years symptom duration) and has already been extensively described elsewhere.[9] In short, inclusion criteria were presence of synovitis determined at physical examination by rheumatologists and symptom duration of <2 years. After inclusion, patient-characteristics, disease activity and laboratory parameters were obtained at baseline, 4-months, 12-months and yearly thereafter by a trained research nurse. Fatigue was assessed yearly with a numeric rating scale (NRS) by a trained research nurse with the question "How tired were you today?" ranging from 0 (no fatigue) to 10 (extreme fatigue).[10] Patients were treated in routine care and in line with (inter-) national recommendations.[11,12] Treating physicians, patients and research nurses were blinded for any MRI data.

From August-2010 onwards MRIs were performed at baseline. RA-patients included in the EAC between August-2010 and March-2020 and fulfilling the 1987- or 2010 criteria within one year were evaluated in the present study (Flowchart in Supplementary 1). In some patients no baseline MRI was performed (mostly due to logistical reasons such as MRI-maintenance) and some baseline MRIs were of insufficient quality (e.g. no contrast enhanced sequences or insufficient fat suppression), implying missingness completely at random. Reassuringly, baseline characteristics were comparable in patients with and without baseline MRI, substantiating this assumption (Supplementary 2). Between 2010 and February-2015 MRIs of RA-patients were repeated at 12 and 24-months. Age, gender, MRI-inflammation and fatigue at baseline were not different between patients included in the periods with or without follow-up MRIs; patients with follow-up MRIs were slightly more often anti-citrullinated protein antibodies (ACPA)-positive (Supplementary 3). Consequently, we studied a representative consecutive sample of 526 early RA-patients that received an MRI at baseline and 199 patients that received follow-up MRIs.

MRI

Wrist, metacarpophalangeal (MCP(2-5)), and metatarsophalangeal (MTP(1-5))-joints on the most painful side at baseline (dominant side in case of symmetric symptoms) were imaged with 1.5TMRI (GE,Wisconsin,USA). Contrast-enhanced T1-weighted FSE fatsat sequences of the wrist and MCP were obtained in all patients. In June-2013, instead of axial T1 and T2-weighted FSE fatsat sequences in the axial plane, contrast-enhanced T1-weighted FSE fatsat sequences in both the coronal and axial plane of the MTPs were added to the protocol. Supplementary 4 provides a detailed scan- and scoring-protocol.

All MRIs were scored for synovitis, tenosynovitis and osteitis in line with RAMRIS and the method of Haavardsholm (Supplementary 4). Baseline MRIs were scored by two experienced readers, blinded to any clinical data. MRIs over time were scored by a single reader, with known time-order, blinded to any clinical data (including DAS and fatigue). Intraclass correlation coefficients were excellent (≥0.95 for total inflammation score; Supplementary 5&6).

Statistical analysis

To study the association of DAS and MRI-inflammation with fatigue at baseline, data was plotted for pairwise analysis and linear regression was used. Because of skewness, the total MRI-inflammation score was log-transformed. Analyses were performed both univariably and corrected for potential confounders: age, gender and ACPA-status. Analyses of MRI-inflammation were not corrected for DAS, because of the collider-effect. Because both MRI-inflammation and fatigue can (indirectly) cause a higher DAS, correction of the relationship of MRI-inflammation and fatigue by the DAS would cause invalid results. This is called a collider effect. The same would be true if we would study the relation between MRI-inflammation and fatigue in patients in DAS-remission. Therefore, these analyses were not performed. This is extensively explained in Supplementary 7.

To assess the association between the 2-year course of DAS and MRI-inflammation with fatigue, change during the first two years was plotted for pairwise analyses. Next, linear mixed models were used, univariably and corrected for the mentioned potential confounders. The mixed models studied the baseline, 1-year and 2-year visit and included a random effect for patient. To allow for robust estimation with few measurements per patient, the residuals and random effects were assumed to be independent. Here again the total MRI-inflammation score was log-transformed and analyses of MRI-inflammation were not corrected for DAS, because of a collider-effect (Supplementary 7).

To study whether decrease in DAS and MRI-inflammation preceded fatigue improvement, cross-lagged models were employed.[13] The influence of decrease in MRI-inflammation on (0-12months) on decrease of fatigue (12-24months) was evaluated corrected for baseline values of the parameters and simultaneous decrease. This was done to evaluate whether excess decrease (e.g. more change than the mean percentual decrease) in inflammation in the first year precedes and predicts excess fatigue decrease in the second year. This was done with the hypothesis that if MRI-inflammation decrease precedes fatigue decrease, MRI-inflammation decrease (0-12months) will associate with fatigue decrease (12-24months) but not the other way around.

The complex structure of the cross-lagged models results in estimates that are not easily interpreted. We therefore expressed them in standardized regression coefficients. Standardized regression coefficients allow for comparison of effect strength between predictors because they are independent of scale. They generally lie between -1 and 1 where a value of -1 (negative) or 1 (positive association) indicates full explanation of the dependent variable by the independent variable and a value of 0 indicates no association.

MRIs at 12-months and 24-months were missing in 20% and 43%, respectively (39 and 86 MRIs, respectively). We assumed that missingness was associated with a measured covariate (e.g. missing at random (MAR) in contrast to missingness completely at random (MCAR)). This was done because patients with less severe disease (as measured by ACPA-positivity) presumably had less follow-up with MRIs. This hypothesis was supported by higher ACPA-positivity (58%) in patients with 24-month MRIs than in patients without (42%;p=0.032) Therefore ACPA was included in the multivariable models, ACPA-stratification was performed and statistical methods appropriate for MAR were employed.

R3.6.3 was used. Two-sided p<0.05 were considered significant.

Sensitivity analyses

Because the associations of the individual DAS components with fatigue might differ, associations of the individual DAS components with fatigue at baseline and over time were assessed.

Because ACPA-positive and ACPA-negative RA are hypothesized to have a difference in underlying pathogenetic mechanisms, this could affect the relationship between inflammation and fatigue. Therefore, we repeated the analyses stratified for ACPA-status.

To ensure our results did not depend on the fatigue measure used and the period assessed, analyses were repeated with two fatigue questions (“Did you feel tired over the last 4 weeks?” and “Did you feel worn out over the last 4 weeks?”) of the short form (SF-)36 as outcome.[10]

Previous research did not show an effect of individual DMARDs on fatigue.[14] However, to ensure our findings over time were not confounded by treatment, we repeated analyses over time in patients receiving initial methotrexate therapy since this was the most frequently occurring initial treatment (69%).

RESULTS

Patient characteristics

526 consecutive RA-patients were studied: mean age was 59, 64% was female, 45% ACPA-positive and median (interquartile range) NRS fatigue was 6 (2-7; Supplementary 2). 199 patients received MRIs during follow-up and had similar baseline characteristics (Supplementary 3). In these patients, NRS fatigue was 6 (2-7) at baseline and decreased slightly to 5 (1-7) at 12-months and 4.5 (1-7) at 24-months.

Association of inflammation and fatigue at diagnosis

We assessed whether patients with more inflammation were more fatigued at diagnosis. RA-patients with more MRI-inflammation were not more fatigued at diagnosis in univariable and multivariable analyses (p=0.08 & p=0.94;Table 1;Figure 1). In contrast, patients with higher DAS were more fatigued at baseline (both p<0.001).

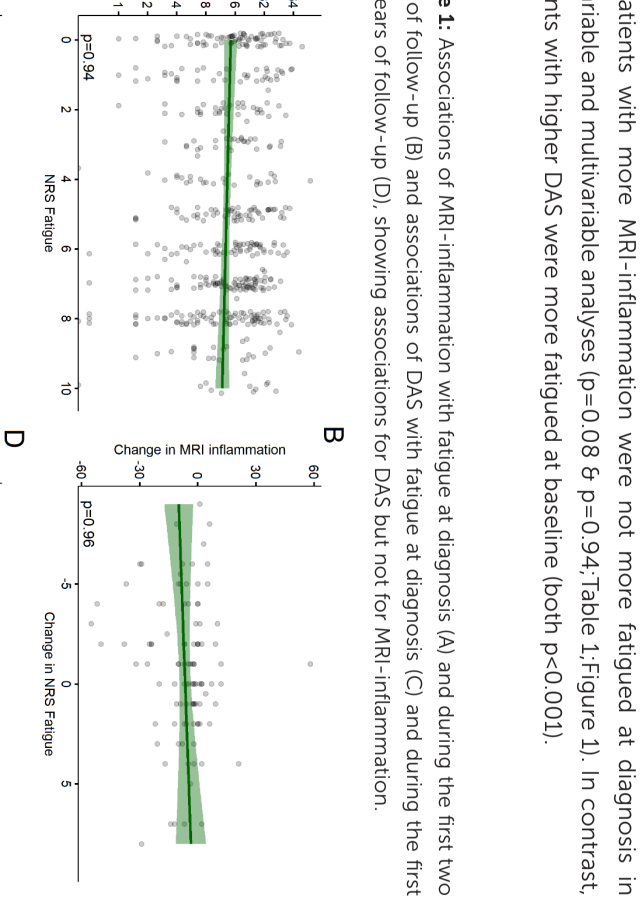

Figure 1: Associations of MRI-inflammation with fatigue at diagnosis (A) and during the first two years of follow-up (B) and associations of DAS with fatigue at diagnosis (C) and during the first two years of follow-up (D), showing associations for DAS but not for MRI-inflammation.

Table 1: Univariable and multivariable associations of MRI-inflammation and DAS at inclusion and fatigue at inclusion and associations of MRI-inflammation and DAS over time with fatigue course over time

Baseline	**Univariable**	**p-value**	**MRI-inflammation corrected for potential confounders**	**p-value**	**DAS corrected for potential confounders**	**p-value**
Total MRI-inflammation	-0.27 (-0.58;0.04)	0.08	-0.01 (-0.36;0.33)	0.94		
Disease activity score	**1.17 (0.80;1.53)**	**<0.001**			**1.22 (0.84;1.59)**	**<0.001**
ACPA	**-0.76 (-1.29;-0.22)**	**0.006**	**-0.80 (-1.33;-0.26)**	**0.004**	**-0.60 (-1.13;-0.09)**	**0.023**
Age in years	**-0.02 (-0.04;0.00)**	**0.031**	-0.01 (-0.04;0.01)	0.16	**-0.02 (-0.04;0.00)**	**0.021**
Female gender	**1.21 (0.67;1.75)**	**<0.001**	**1.21 (0.65;1.76)**	**<0.001**	**1.04 (0.50;1.58)**	**<0.001**
Over time	**Univariable**	**p-value**	**MRI-inflammation corrected for potential confounders**	**p-value**	**DAS corrected for potential confounders**	**p-value**
Total MRI-inflammation	-0.01 (-0.29;0.27)	0.95	-0.01 (-0.30;0.28)	0.96		
Disease activity score	**1.03 (0.78;1.28)**	**<0.001**			**1.01 (0.77;1.26)**	**<0.001**
ACPA	0.10 (-0.56;0.76)	0.77	0.09 (-0.58;0.76)	0.79	-0.06 (-0.66;0.55)	0.86
Age in years	-0.02 (-0.04;0.01)	0.16	0.00 (-0.03;0.03)	0.95	-0.02 (-0.04;0.00)	0.13
Female gender	**1.27 (0.59;1.94)**	**<0.001**	**1.49 (0.78;2.20)**	**<0.001**	**0.79 (0.15;1.43)**	**0.016**

Legend: ACPA: anti-citrullinated protein antibodies; DAS: disease activity score; bold results indicate p<0.05.

Legend: NRS, numeric rating scale. The y-axis in (A) log-transformed. NRS fatigue values in (A&C) were jittered along the x-axis with a width of 0.2 at either side

Association of course of inflammation and fatigue

The association between the time-courses of inflammation and fatigue during the first 2 years of the disease was assessed. The course of MRI-inflammation was not associated with that of fatigue (p=0.95&p=0.96; Table 1;Figure 1). However, DAS decrease was associated with simultaneous fatigue decrease (both p<0.001)

Time orders in decrease of inflammation and fatigue

We hypothesized that decrease in inflammation can precede fatigue improvement and therefore relatively more inflammation decrease would associate with relatively more subsequent fatigue decrease but not vice versa. In line with our hypothesis, MRI-inflammation decrease preceded fatigue decrease (p=0.039;Table 2), but fatigue decrease did not precede MRI-inflammation decrease (p=0.63).

In concordance with MRI-inflammation, DAS decrease 0-12 months preceded fatigue decrease 12-24 months (p=0.012) but fatigue decrease did not precede DAS decrease (p=0.23). The effect-strength of MRI-inflammation was similar, but not stronger, to than of DAS.

Table 2: Estimates of subsequent change of MRI-inflammation, DAS and fatigue

Subsequent change	Standardized regression coefficient	P-value
MRI-inflammation precedes fatigue	**0.17 (0.01;0.34)**	**0.039**
Fatigue precedes MRI-inflammation	0.04 (-0.12;0.20)	0.63
DAS precedes fatigue	**0.19 (0.04;0.34)**	**0.012**
Fatigue precedes DAS	0.10 (-0.07;0.27)	0.23

Legend: Analyses were performed with the hypothesis that if inflammation decrease precedes fatigue decrease, inflammation decrease (0-12m) will associate with fatigue decrease (12-24m) but not the other way around. Standardized regression coefficients of change of one inflammatory feature to subsequent change in another inflammatory feature, corrected for the simultaneous pattern and previous values of those inflammatory features, with 95% confidence intervals. bold: significant estimate (p<0.05). DAS: disease activity score.

Sensitivity analyses

The associations of the individual DAS components with fatigue were assessed (Supplementary 8). At baseline, the visual analogue scale for general health (VAS) and the tender joint count (TJC) associated with fatigue. Over time, also the swollen joint count (SJC) and erythrocyte sedimentation rate (ESR) associated with fatigue in univariable but not in multivariable analyses.

Analyses were repeated stratified for ACPA-status (Supplementary 9). Similar results were obtained, except that time-orders of fatigue and inflammation decrease did not attain statistical significance in ACPA-negative patients.

To ensure robustness of results, independent of the fatigue measure used and time period assessed, analyses with two questions of the SF-36 were performed (Supplementary 10). These yielded similar results except that time orders of decrease in MRI-inflammation and fatigue were not identified.

Sensitivity analyses over time in patients receiving initial methotrexate yielded similar results except that the association of MRI-inflammation decrease and subsequent fatigue decrease did not attain statistical significance (Supplementary 11).

DISCUSSION

Fatigue is an important contributor to disease burden in RA. It is considered a consequence of inflammation. Although the association of DAS with fatigue is extensively studied, the contribution of joint inflammation in the hands and feet, as detected by very sensitive imaging techniques, was unexplored. We observed that higher clinical disease activity was associated with more fatigue, both at the time of diagnosis and during the disease course. This confirms previous studies. In contrast, MRI-inflammation was not helpful in explaining fatigue. This supports the concept that fatigue in patients with classified RA is partly disconnected from inflammation and caused by other processes.

We observed that decrease of MRI-inflammation in year-1 preceded subsequent fatigue decrease in year-2. However it most likely did not aid in explaining fatigue on top of the DAS, because standardized regression coefficients were similar, indicating that these inflammation indicators explained similar variance in subsequent fatigue. Moreover, the observed association of MRI-inflammation decrease on subsequent improvement in fatigue was not observed in sensitivity analyses, indicating that this finding was less robust. Therefore, the results on the added value of imaging detected joint inflammation in the hands and feet in explaining fatigue in this study are rather negative.To further investigate which kind of inflammation best explains fatigue in RA, we assessed the association of the individual DAS components with fatigue. We found that the subjective markers of inflammation (VAS, TJC) better associated with fatigue and that the objective markers (SJC, ESR) did not associate with fatigue at baseline and in multivariable analyses. This might partly explain the absence of association of MRI-inflammation and fatigue as MRI-inflammation is a very objective measure of inflammation.

While MRI-inflammation is measured locally in small joints that are preferentially affected in RA, the DAS also includes more comprehensive joint counts as well as systemic measures of inflammation such as the ESR. Our aim was to investigate whether local joint inflammation as measured sensitively with MRI could aid in

explaining fatigue in RA. Despite this hypothesis, we found no association between MRI-detected joint inflammation and simultaneously present fatigue. Therefore we feel that the data suggest that fatigue is partly unexplained by inflammation, even when regular measures of inflammation and imaging detected inflammation are both considered. While clinical trials are needed to confirm this, the findings from the present observational study imply that treating MRI-inflammation does not result in lower fatigue. This is in contrast to treating clinical disease activity as measured by the DAS, which is associated with fatigue over time. This is also confirmed by a trial that failed to show an effect on fatigue when treatment was aimed at imaging remission instead of DAS remission.[15] Altogether, treatment strategies to lower fatigue should be aimed at the DAS or clinical remission and not at imaging detected inflammation.

We found that fatigue is partly unexplained by clinical inflammation and MRI-inflammation. This implies that it is partly disconnected from inflammation and might have become chronic by itself or might be caused by other processes such as depression and/or secondary fibromyalgia. Unfortunately, we were not able to assess these factors in the current study. Further research can help to elucidate into what extent fatigue in RA can be explained by these factors or has become chronic by itself. We reconfirmed the findings that showed that a decrease in DAS associated with a simultaneous decrease in fatigue and with subsequent decrease in fatigue.[6,7] Moreover, our study is the first to study time orders of MRI-inflammation decrease and fatigue decrease. In these analyses, time order of decrease implies the directionality of causality. Still, fatigue during disease course remains largely unexplained by inflammation, which implicates that fatigue has become chronic and possibly a separate disconnected process already very early in the disease-course. It remains to be studied whether MRI-inflammation in a pre-arthritis phase has a stronger connection with fatigue and whether intervention with DMARDs in the phase when disease processes are less mature is more effective in treating fatigue.

Our results were similar for RA-patients with and without ACPA. Both subsets of RA have differences in the underlying pathogenesis, with the adaptive immune response, and B-cells in particular, playing a more prominent role in the autoantibody positive RA. B-cell depletion has been proposed as a treatment for chronic fatigue.[16] If these cells would play an important role in fatigue in RA, it could be expected that ACPA-positive patients would have more severe fatigue at the time of diagnosis and that treatment could affect the response in fatigue differently in both ACPA-subsets. In contrast to this reasoning, in our data, ACPA-positive patients were less fatigued at baseline, similarly fatigued during the disease course and effects of inflammation on fatigue during disease course were also similar. This might argue against a major role for the B-cell response in fatigue in RA.

Unfortunately we could not study the effect of MRI-inflammation on fatigue in addition to the DAS due to a collider effect. However, since we found no effect of MRI-inflammation on simultaneous fatigue, it is unlikely that MRI-inflammation could explain simultaneous fatigue independent of the DAS. This is supported by previous finding that US remission in addition to DAS remission was not associated with lower fatigue.[17]

A limitation is that some MRIs were missing during follow-up. We assumed this missingness to be associated with disease severity (e.g. missingness at random/MAR) which was confirmed by more ACPA-positivity in patients completing 2-year MRIs. Statistical techniques appropriate for MAR were employed and correction for variables associated with disease severity (ACPA-status) was performed. This correction did not substantially alter our results, implying that missingness associated with disease severity did not substantially influence our univariable results. While, to our knowledge, this study was performed on the largest observational longitudinal database of MRI scans in RA, the number of missing MRIs during follow-up remains an important limitation.
In this study, we evaluated unilateral MRI-inflammation of the hands and feet of the most affected side. MRI-inflammation could also be present at other joint regions and in the preferential regions it is often bilaterally present. Reassuringly, previous literature showed that bilateral scanning conveyed little additional information compared to scanning of only the most affected side.[18,19] Although it is a limitation that not more joints were imaged, we think it is unlikely that it majorly affected our results.

We measured fatigue severity with a single item fatigue measure, the NRS: "How tired were you today?".[10] This measure is simple to administer and also has a good construct validity, sensitivity to change, and test-retest properties in RA. Many other multi-item or multidimensional fatigue measures are available and are able to explore various domains of fatigue.[10] Those multidomain scores can aid in understanding fatigue causality or the effect of fatigue-specific interventions. We repeated analyses with two items of the SF-36 that assesses a longer period of time (4 weeks) and obtained similar results.[10] Despite that separate items of the SF-36 have not been validated, this allowed us to associate MRI-inflammation with multiple reliable and easily interpretable measures of fatigue over a short and a long period. These analyses showed the robustness of our results.

In conclusion, in the first large observational study evaluating the relation between MRI detected joint inflammation and fatigue in RA, we showed that measuring inflammation with MRI does not aid in explaining fatigue in RA. The results imply that aiming at imaging remission does not lower fatigue in RA. Consequently treatment strategies to lower fatigue should be aimed at the DAS, as shown in previous studies. Fatigue

in RA is partly disconnected from inflammation and may have become chronic by itself. Other types of interventions may be explored to reduce the burden of fatigue in patients diagnosed with RA. However, it remains to be determined if imaging detected inflammation is more strongly connected to fatigue in phases that precede clinical arthritis, and if DMARD-treatment in this phase is more effective in reducing fatigue or preventing chronic fatigue.

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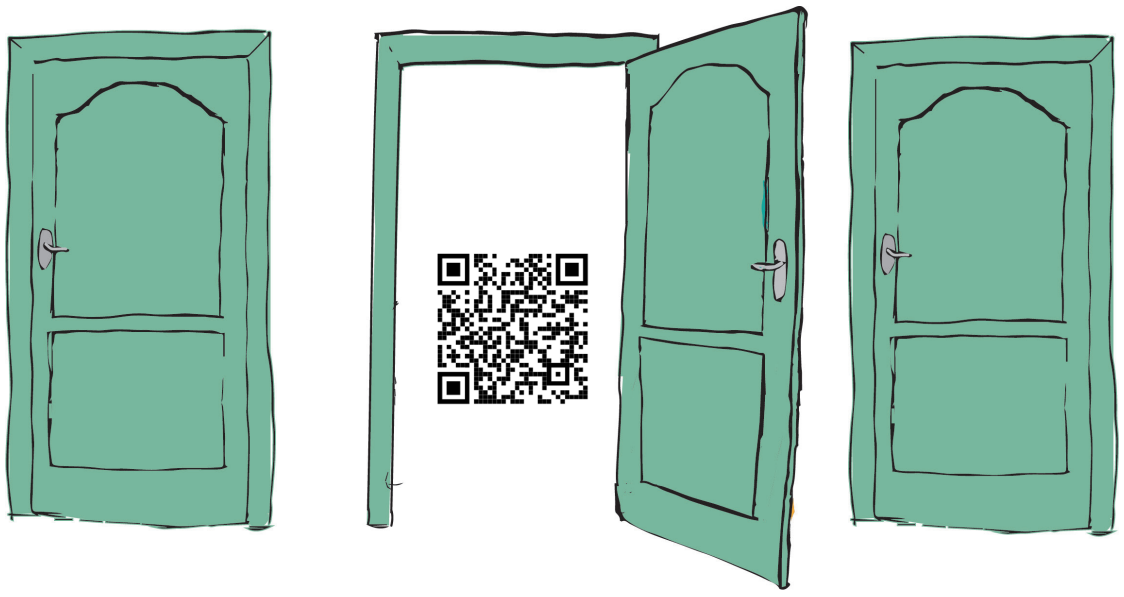

CHAPTER 6

Differing time orders of inflammation decrease between ACPA-subsets in RA-patients suggest differences in underlying inflammatory pathways

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ABSTRACT

Objectives

Advanced imaging modalities have shown that not only joints but also bones and tendon sheaths can be inflamed at diagnosis of rheumatoid arthritis. We aimed to better understand the time order in which the inflamed tissues respond to DMARD-treatment. Also, because ACPA-status may reflect a different pathophysiology, differences in time order of inflammation decrease were hypothesized between these disease types.

Methods

216 consecutive patients presenting with rheumatoid (n=176) or undifferentiated arthritis (N=40), who all started with csDMARD-treatment, were studied. 1.5T contrast-enhanced hand and foot MRIs were performed before treatment and after 4, 12 and 24-months. Cross-lagged models evaluated the influence of two time-patterns: a simultaneous pattern ("change in one inflammatory feature associated with change in another feature") and a subsequent pattern ("change in one inflammatory feature preceded change in another feature"). ACPA-stratification was performed.

Results

The median symptom duration at presentation was 13 weeks. 44% of patients was ACPA-positive.

All pairs of inflammatory features decreased simultaneously in all time-intervals (0–4/4–12/12–24m; p<0.05). Moreover, time orders were identified: synovitis decrease preceded tenosynovitis decrease (0-4m->4-12m; p=0.02 & 4-12m->12-24m; p=0.03). Largely similar results were obtained in both ACPA-subgroups. Additionally, in ACPA-positive but not ACPA-negative patients, synovitis decrease preceded osteitis decrease (4-12m->12-24m; p=0.002).

Conclusion

This study increased the understanding of the response to treatment on tissue level. Additional to simultaneous decrease of inflammation, synovitis decrease preceded tenosynovitis decrease. Differences in time order of inflammation decrease between ACPA-subgroups suggest differences in underlying inflammatory pathways.

KEY MESSAGES

- This study increased the understanding of the response to treatment on tissue level.
- Additional to simultaneous decrease of inflammation, synovitis decrease preceded tenosynovitis decrease.
- Differences in time order of inflammation decrease between ACPA-subgroups suggest differences in underlying inflammatory pathways.

BACKGROUND

During the last decennium advanced imaging modalities, including MRI, have refined our understanding of the tissues involved in rheumatoid arthritis (RA) and have shown that not only joints but also bones and adjoining synovial tendon sheaths of small joints are frequently inflamed.(1,2) These tissues are distinct anatomical structures but synovitis, osteitis and tenosynovitis frequently co-occur at diagnosis.(1,3) Remarkably, previous research suggested time-orders in inflammation development of these tissues during RA-development.(2,4) If time-order are present in developing RA, we assume that there are also time-orders in inflamed tissue in decrease of inflammation. However, little is known about the mutual influence of inflammation of these tissues when inflammation is resolving due to treatment.

Some studies investigated inflammation decrease in joints, bones and tendon sheaths after treatment in early RA.(5-7) However, they did not determine whether inflammation decrease is simultaneous in all tissues or whether sequences also play a role, as time-orders were not studied. Also, anti-citrullinated protein antibody (ACPA)-subgroups were not studied separately, while these are considered different disease types with differences in underlying pathophysiology.(8-10) Consequently, differences in time-order of inflammation decrease in response to treatment can be expected but, to our knowledge, this has not been explored yet.

Our aim was to achieve a better understanding of the time-orders in which the different inflamed tissues (joint, bone, tendon sheath) respond to DMARD-treatment, and whether this differs between ACPA-subgroups. In the Leiden Early Arthritis inception cohort (EAC), MRIs of undifferentiated arthritis (UA) and RA-patients were performed at presentation (before DMARD-initiation) and after 4, 12 and 24-months. This allowed for differentiation between simultaneous and subsequent patterns of inflammation decrease of joint, bone and tendon sheath after DMARD-initiation in 3 consecutive time periods.

METHODS

Patients

Since 1993, consecutive early arthritis patients (<2years symptom duration) were included in the Leiden EAC. This inception cohort is extensively described elsewhere.(11) In short, patient-characteristics, disease activity and laboratory parameters were obtained at baseline, 4-months, 12-months and yearly thereafter. From August-2010 until February-2015, MRIs were performed at baseline and 4, 12 and 24-months when the initial clinical diagnosis was UA or RA.

Treatment

Patients were treated in routine care and in line with (inter-)national guidelines.(12) Medication data were extracted from the hospital patient information system and quality controlled. Doctors and patients were blinded for MRI-data.

Patient selection

From all patients with an initial clinical diagnosis of RA or UA were consecutively included from August-2010 until February-2015 (n=655) patients starting with DMARDS (including glucocorticoids) within 100-days after the first rheumatology outpatient clinic visit were selected (n=376). 160 patients did not undergo repeated MRIs (mostly for logistical reasons), resulting in 216 patients that were studied. Baseline characteristics of patients who started early with DMARD-treatment and who did and did not have repeated MRIs were not statistically significantly different (Supplementary1).

MRI

MRI was performed at baseline (before DMARD-initiation) and 4, 12 and 24-months. Metacarpophalangeal (MCP(2-5)), wrist and metatarsophalangeal (MTP(1-5))-joints on the most painful side at baseline (dominant side in case of symmetric symptoms) were imaged with 1.5TMRI (GE,Wisconsin,USA). Follow-up MRIs were performed at the side of the baseline MRI. MRIs were scored for synovitis and osteitis in line with RAMRIS and tenosynovitis as described by Haavardsholm, by one reader, with known time-order, blinded for any clinical data.(13,14) Intrareader reliability was excellent (ICC0.98; Supplementary2). Scores were summed per inflammatory feature per patient. Supplementary3 provides a detailed scan and scoring protocol.

Statistical analysis

Data of three time-intervals (0–4/4–12/12–24-months) were studied with cross-lagged models.(15) Cross-lagged models can evaluate the influence of two time-patterns in one model: 1) a simultaneous pattern ("change in one inflammatory feature is associated with change in another feature") and 2) a subsequent pattern ("change in one inflammatory feature precedes change in another feature") as is shown in Supplementary4. Despite these benefits, these models are infrequently used in rheumatology research and most often employed in psychology.(15) Further explanation is presented in Supplementary5.

Because of skewness, MRI-variables were log-transformed, after addition of 1 point to facilitate transformation of zeroes. This and the complex structure of the cross-lagged

models results in estimates that are not easily interpreted. We therefore expressed them in standardized regression coefficients and correlations. Standardized regression coefficients are independent of scale and lie between -1 and 1. A value of -1 (negative) or 1 (positive association) indicates full explanation of the dependent variable by the independent variable and a value of 0 indicates no association. Congruently, correlations lie between -1 and 1, and 0 indicates no association.

MRIs at 4-months, 12-months and 24-months were missing in 11%, 20% and 47%, respectively (23, 44 and 102 MRIs, respectively). We assumed missing at random (MAR), not missing completely at random, because patients with a less severe disease presumably had less follow-up with MRI. MAR implies that missingness, not explained by variables included in the model, is random. Since disease activity is correlated with MRI-inflammation,(16) which is included in the model, and ACPA-stratification was performed, no further variables associated with missingness were included in the models to achieve MAR. Also, cross-lagged models were fitted with full-information likelihood, appropriate for MAR.(17)

Because ACPA-status may reflect a different pathophysiology, analyses were repeated stratified for ACPA-status (anti-CCP2).

Additional analyses

As sensitivity analyses, analyses were repeated in the subgroup of RA-patients (clinical diagnosis plus fulfilment of 1987- or 2010-criteria <1-year). In addition, analyses were repeated in patients that started DMARD-treatment within 31-days.

To assess the influence of initial treatment, sensitivity analyses were performed in patients starting methotrexate as first therapy (as this was the most frequently used first-line DMARD). In addition, analyses were repeated in patients starting methotrexate without corticosteroid bridging.

To assess natural course, decrease of MRI-inflammation of UA and RA-patients that, in contrast to the guidelines,(12) did never receive DMARD-treatment and were therefore excluded, was presented.

R3.6.1, RStudio1.2.5001, Onyx 1.0-101 and OpenMx 2.14.11 were used (Supplementary5). Two-sided p-values <0.05 were considered significant.

RESULTS

Baseline characteristics

Patient baseline characteristics are shown in Supplementary6: mean age was 58, 62% female, 44% ACPA-positive, 74% received initial methotrexate and the remaining patients started with other csDMARDs. The median symptom duration at presentation was 13 weeks and the median time to DMARD-start 2.4 weeks. 82% classified as RA (Supplementary7).

Simultaneous and subsequent patterns

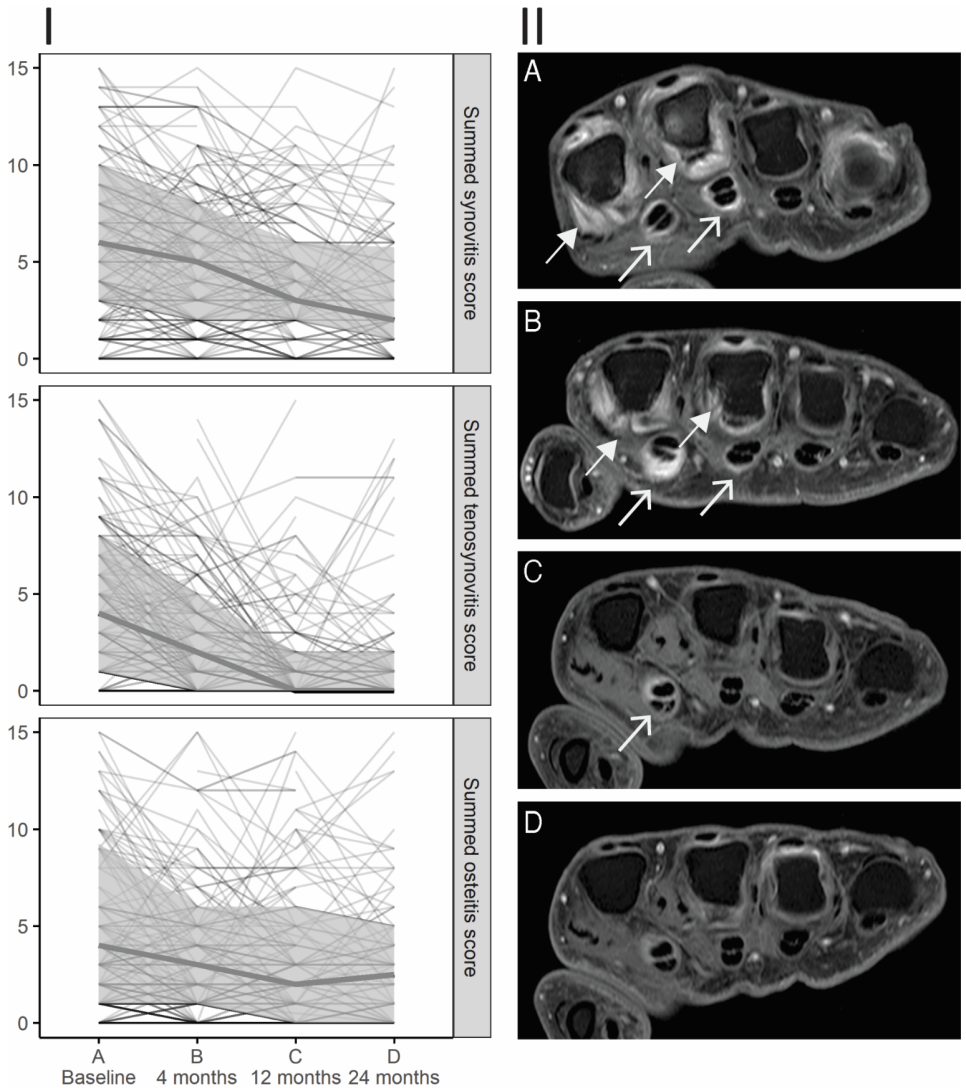
Plotting the MRI-data over time revealed that synovitis, osteitis and tenosynovitis decreased during follow-up (Figure 1). For osteitis, this decrease manifested predominantly in decreasing interquartile ranges.

To assess the influence of both the simultaneous and subsequent pattern in one model, cross-lagged models were used. With respect to the simultaneous patterns, all pairs of inflammatory features showed significant simultaneous decrease in all time-intervals (0–4/4–12/12–24m (months);Table 1).

In addition to simultaneous decrease, time-orders were identified (Table 1). Predominantly, synovitis decrease preceded tenosynovitis decrease. Synovitis decrease 0-4m preceded tenosynovitis decrease 4–12m (standardized regression coefficient (β) and 95% confidence interval: 0.28(0.04;0.53); Figure 1) and synovitis decrease 4-12m preceded tenosynovitis decrease 12-24m (β=0.27(0.04;0.50)).

Moreover, early tenosynovitis decrease (0-4m) significantly preceded osteitis decrease 4-12m with a smaller effect size (β=0.15(0.00;0.31)). However, 'late' tenosynovitis decrease (4-12m) did not precede osteitis decrease 12-24m (β=0.01(-0.13;0.14)), together this suggests that this finding with a smaller effect size is less robust than the other findings.

Figure 1: Individual courses of synovitis, tenosynovitis and osteitis in all patients studied (I) and an example of serial MRI of the MCP-joints of an individual patient (II) at (A) baseline, (B) 4 months , (C) 12 months and (D) 24 months



Legend: Part I: Lines represent individual patient trajectories. The bold line represents the median and the grey area the interquartile range. For readability, summed RAMRIS scores above 15 were omitted from the graph; Part II: These MRIs show synovitis (closed arrows) decrease between 0 and 4 months preceding tenosynovitis (open arrows) decrease between 4 and 12 months

Table 1: Estimates of simultaneous and subsequent change of three inflammatory features

Simultaneous change		**All patients**	**ACPA-positive**	**ACPA-negative**
Synovitis with Tenosynovitis				
	0-4m	0.20 (0.14;0.26)*	0.21 (0.12;0.31)*	0.20 (0.12;0.28)*
	4-12m	0.20 (0.13;0.28)*	0.19 (0.09;0.30)*	0.22 (0.11;0.33)*
	12-24m	0.29 (0.20;0.38)*	0.27 (0.15;0.39)*	0.31 (0.18;0.45)*
Synovitis with Osteitis				
	0-4m	0.13 (0.08;0.19)*	0.19 (0.10;0.28)*	0.10 (0.02;0.17)*
	4-12m	0.16 (0.09;0.22)*	0.14 (0.05;0.23)*	0.17 (0.07;0.26)*
	12-24m	0.11 (0.04;0.19)*	0.20 (0.10;0.30)*	0.00 (-0.09;0.09)
Tenosynovitis with Osteitis				
	0-4m	0.07 (0.01;0.14)*	0.06 (-0.03;0.16)	0.08 (-0.01;0.17)
	4-12m	0.13 (0.05;0.22)*	0.11 (0.01;0.22)*	0.21 (0.09;0.33)*
	12-24m	0.12 (0.04;0.21)*	0.14 (0.03;0.25)*	0.07 (-0.05;0.19)

Subsequent change	**All patients**	**ACPA-positive**	**ACPA-negative**
Synovitis precedes Tenosynovitis			
0-4m -> 4-12m	0.28 (0.04;0.53)*	0.23 (-0.11;0.56)	0.35 (0.01;0.68)*
4-12m -> 12-24m	0.27 (0.04;0.50)*	0.38 (0.10;0.66)*	0.18 (-0.17;0.54)
Tenosynovitis precedes Synovitis			
0-4m -> 4-12m	0.04 (-0.11;0.19)	0.08 (-0.13;0.29)	0.02 (-0.20;0.23)
4-12m -> 12-24m	0.04 (-0.13;0.20)	0.08 (-0.18;0.34)	-0.03 (-0.23;0.17)
Synovitis precedes Osteitis			
0-4m -> 4-12m	0.11 (-0.09;0.32)	0.13 (-0.16;0.42)	0.07 (-0.22;0.36)
4-12m -> 12-24m	0.09 (-0.09;0.27)	0.40 (0.17;0.64)*	-0.23 (-0.45;-0.01)*
Osteitis precedes Synovitis			
0-4m -> 4-12m	0.12 (-0.04;0.27)	0.08 (-0.15;0.32)	0.13 (-0.08;0.33)
4-12m -> 12-24m	0.17 (-0.05;0.38)	0.24 (-0.05;0.53)	0.16 (-0.14;0.47)
Tenosynovitis precedes Osteitis			
0-4m -> 4-12m	0.15 (0.00;0.31)*	0.04 (-0.18;0.25)	0.19 (-0.02;0.40)
4-12m -> 12-24m	0.01 (-0.13;0.14)	0.12 (-0.11;0.35)	-0.11 (-0.27;0.04)
Osteitis precedes Tenosynovitis			
0-4m -> 4-12m	-0.02 (-0.23;0.19)	-0.02 (-0.31;0.26)	-0.04 (-0.33;0.25)
4-12m -> 12-24m	0.14 (-0.10;0.39)	0.23 (-0.07;0.54)	0.09 (-0.30;0.49)

Legend: Estimates of simultaneous change represent correlation of proportion of change of two inflammatory features that is not explained by the subsequent pattern and previous values of those inflammatory features, with 95% confidence intervals. Estimates of subsequent change represent standardized regression coefficients of change of one inflammatory feature to subsequent change in another inflammatory feature, corrected for the simultaneous pattern and previous values of those inflammatory features, with 95% confidence intervals. Standardized regression coefficients are independent of scale and lie between -1 and 1. A value of -1 or 1 indicates full explanation of change in one inflammatory feature by change in the previous period of another inflammatory feature and a value of 0 indicates no explanation. Values -1 and 0 (negative estimate) indicate that a decrease in the first period is associated with less decrease in the subsequent period, in addition values between 0 and 1 indicate that a decrease in the first period is associated with more decrease in the subsequent period. *: significant estimate (p<0.05)

ACPA-stratification

Simultaneous decrease was present in both ACPA-subsets and similar to that described above (Table 1).

Also in both ACPA-subsets synovitis decrease preceded tenosynovitis decrease with similar estimates, albeit not always reaching statistical significance which may be due to the smaller sample size (Table 1).

In addition, an ACPA-specific time-order was identified: In ACPA-positive patients synovitis decrease 4-12m preceded osteitis decrease 12-24m (β=0.40(0.17;0.64)). This was significantly different from ACPA-negative patients (p<0.001), in which the estimate was in the opposite direction (β=-0.23(-0.45;-0.01)).

Additional analyses

All analyses were repeated in RA-patients (n=176) and in patients that started DMARD-treatment within 31-days (n=153); similar results were obtained (Supplementary8&9). In patients starting with methotrexate, similar results were obtained, also when excluding patients receiving corticosteroid bridging (Supplementary10).

Finally the natural course of subgroup of patients, UA and RA-patients that did never receive DMARD-treatment (and were therefore excluded from the analyses)), was plotted and showed little decrease (Supplementary11).

DISCUSSION

We aimed to better understand the time-order of the response of different inflamed tissues (joint, bones and adjoining tendon sheaths of small joints) to DMARD-treatment. Using cross-lagged models, we found that the inflammatory features not only decrease simultaneously but also that decrease in synovitis preceded decrease in tenosynovitis.

Since the last decade advanced imaging studies have revealed that inflammation in RA is not only synovitis but also comprises osteitis and tenosynovitis. Information on time-orders of inflammation decrease provide insight in the sensitivity to treatment of these different inflamed tissues. Previous research on RA-development suggested that tenosynovitis presents early in the pre-arthritis phase and is followed by synovitis.(2,4) Our research suggests that a decrease of synovitis is followed by a decrease in tenosynovitis; these findings together possibly suggest that inflammation that comes the earliest (e.g. tenosynovitis), resolves slower. Further research is needed to elucidate the molecular mechanism of this relationship.

Previous studies have shown that osteitis is more often present in ACPA-positive RA and is strongly associated with erosion development and is therefore an important feature in ACPA-positive RA.(18,19) In our data ACPA-positive patients at baseline had slightly higher osteitis scores (Supplementary7). Moreover our data further supports that osteitis is an important feature in ACPA-positive RA by showing that synovitis decrease 4-12m preceded subsequent osteitis decrease 12-24m only in ACPA-positive patients. In contrast to this late subsequent decrease, no significant effect of synovitis decrease 0-4m on osteitis decrease 4-12m was observed in ACPA-positive patients. This could indicate that suppression of inflammation in ACPA-positive patients affects synovitis first, but that a prolonged suppression of inflammation is needed to attain osteitis decrease in these patients.

In ACPA-negative patients, the effect of synovitis decrease 4-12m on subsequent osteitis decrease 12-24m was negative, meaning that more decrease in synovitis 4-12m is associated with less decrease in osteitis 12-24m. In addition, synovitis and osteitis showed high simultaneous decrease in 4-12m. Together, this can imply that more inflammation suppression and resulting synovitis and osteitis decrease between 4-12m results in a plateau in osteitis 12-24m in ACPA-negative patients.

To our knowledge, our study is the first to show a differential disease course after treatment at the tissue level in ACPA-subgroups. While this might not have any direct clinical implications, important improvements of treatment are often fuelled by a better understanding of the pathophysiology of disease. By increasing knowledge of the effect of treatment of RA on tissue level, stratified for autoantibody-status, we ultimately hope to contribute to improved treatment in RA, that might differ between ACPA-subgroups.

This study is, to our knowledge, the first observational MRI-study in DMARD-naïve patients that includes both early (<6m) and late (>1y) MRIs. Timing of MRIs was set at fixed timepoints after inclusion and therefore not dependent on date of DMARD-initiation. Reassuringly, in patients treated within 31-days, therefore having similar time periods between treatment and MRIs, results were comparable. The second MRI was made after 4-months, the time when the efficacy of the initiated conventional DMARD is generally evaluated. Therefore, we could not perform analyses on very fast inflammation decrease due to corticosteroids. This was beyond the scope of this study. Limitations include that MRI scans were scored by a single reader. Encouragingly, intrareader reliability was excellent (Supplementary2). Moreover, two different MRI-protocols were used for the MTP-joints. Reassuringly, previous studies showed that

these protocols perform equally in depicting osteitis and sensitivity analyses omitting the MTPs showed similar results (Supplementary3).(20) The number of patients with missing MRI increased over time, especially in patients with less severe disease, resulting in missingness depending on measured covariates (MAR). Hence, we used statistical techniques appropriate for MAR.

Numbers became smaller after ACPA-stratification. Therefore, main analyses were performed in all patients with both definite RA and UA that required, according to the rheumatologist, early DMARD-treatment. Several sensitivity analyses were performed to assess robustness of results, all showing similar results. Additionally, data were insufficient to perform analyses on joint level. Therefore, validation of our findings in larger longitudinal MRI-studies in both ACPA-subgroups is warranted.

Our analyses were conducted in longitudinal cohort data, not in randomized placebo controlled trial data. While treatment was not randomized, it was protocolized, indicated by >80% of RA-patients starting with initial methotrexate. Analyses in patients starting with methotrexate showed similar results. Analyses for patients that started with other conventional first-line DMARDs were not performed due to low numbers. Biologicals were only allowed if patients failed on ≥2 conventional DMARDs and biologic use during the studies 2-year follow-up was infrequent (3% in ACPA-negative and 14% in ACPA-positive patients at year 2), impeding sensitivity analyses in this group. Therefore, whether different DMARDs (including biologicals) have differential influence on the tissue level remains an interesting question for future research.

Importantly both patients and rheumatologists were blinded for MRI-data, limiting the influence of MRI-inflammation on treatment decisions. Still, inflammation decrease can be partly due to natural course or bias due to reading MRIs in chronological order. To evaluate this, MRIs of UA and RA-patients that, in contrast to the guidelines,(12) did never receive DMARD-treatment, were scored simultaneously with the MRIs of our study, blinded for clinical data. This revealed that MRI-inflammation decreased little in untreated patients (Supplementary11). Therefore, the decrease observed in the treated patients most likely represents a treatment-effect.

In conclusion, this study increased the understanding of treatment-response on tissue level. In addition to simultaneous decrease of synovitis, osteitis and tenosynovitis, time-orders of response in inflamed tissues were identified, that were partly different in the ACPA-subgroups. This suggests different inflammatory pathways underlie MRI-inflammation in ACPA-positive and ACPA-negative disease.

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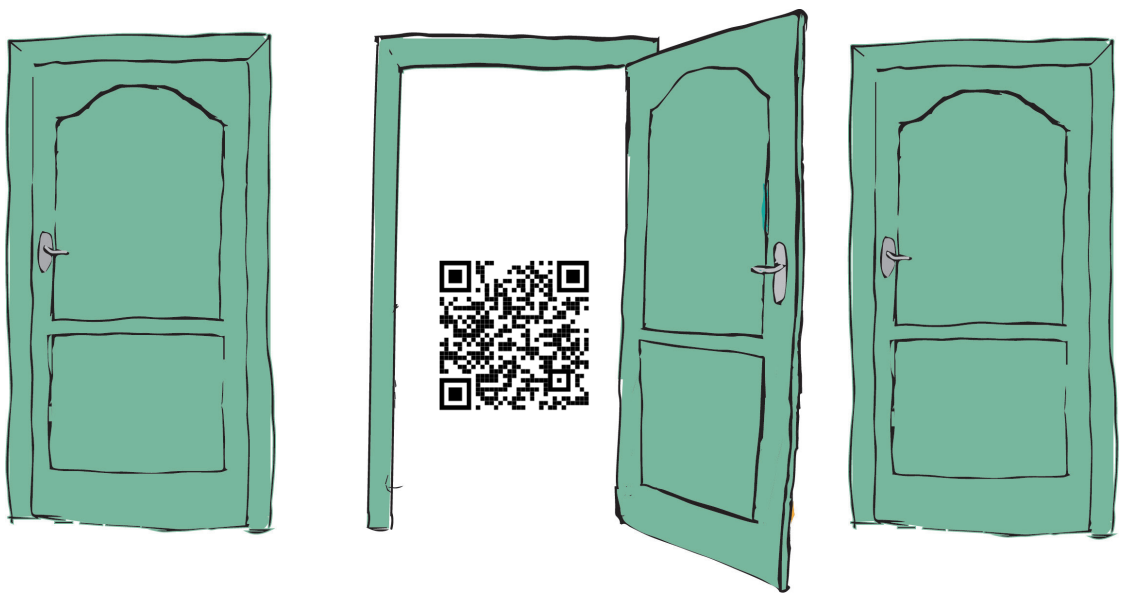
6

LONG-TERM OUTCOMES



CHAPTER 7

Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: a longitudinal cohort study in the Netherlands

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ABSTRACT

Background

Based on different genetic and environmental risk factors and histology, it has been proposed that rheumatoid arthritis (RA) consists of two types: autoantibody-positive and autoantibody-negative RA. However, until now, this remained hypothetical. To assess this hypothesis, we studied whether the long-term outcomes differed for these two groups of RA-patients.

Methods and Findings

In the Leiden Early Arthritis Cohort, 1285 consecutive RA-patients were included between 1993-2016 and followed yearly. Treatment protocols in routine care improved over time, disregarding autoantibody-status, 5 inclusion periods were used as instrumental variables: 1993-1996 delayed mild disease modifying anti-rheumatic drug (DMARD) initiation (reference period); 1997-2000 early mild DMARDs; 2001-2005 early methotrexate; 2006-2010 early methotrexate followed by treat-to-target adjustments; 2011-2016 similar to 2006-2010 plus additional efforts for very early referral.

Three long-term outcomes were studied: SFDR (persistent absence of clinical synovitis after DMARD-cessation), mortality and functional disability measured by yearly health assessment questionnaires (HAQ). Treatment response on the short-term (disease activity) was measured by DAS28-ESR. Linear mixed models and Cox regression were used, stratified for autoantibody-positivity, defined as IgG anti-CCP2 and/or IgM rheumatoid factor-positivity.

823 patients had autoantibody-positive RA (mean age 55, 67% female); 462 patients autoantibody-negative RA (age 60, 64% female). Age, gender and percentage of autoantibody-positive patients were constant throughout the inclusion periods.

Disease activity significantly decreased over time within both groups. SDFR-rates increased since introduction of treat-to-target (HR 2006-2010: 3.35 [1.46 to 7.72; p=0.004] & HR 2011-2016: 4.57 [1.80 to 11.6; p=0.001]) in autoantibody-positive RA, but not in autoantibody-negative RA. In autoantibody-positive RA, mortality decreased significantly since treat-to-target treatment-adjustments (HR 2006-2010: 0.56 [0.34 to 0.92; p=0.023] & HR 2011-2016: 0.33 [0.14 to 0.77; p=0.010]), but not in autoantibody-negative RA (HR 2006-2010: 0.79 [0.40 to 1.56; p=0.50] & HR 2011-2016: 0.36 [0.10 to 1.34; p=0.13]). Similarly, functional disability improved in autoantibody-positive RA since 2001-2005 (range -0.16 [-0.29 to -0.03; p=0.043] to -0.32 [-0.44 to -0.20; p<0.001]) units improvement), but not in autoantibody-negative RA (range 0.10 [-0.12 to 0.31; p=0.38] to -0.13 [-0.34 to 0.07; p=0.20]) units improvement). Limitations to note were that treatment was not randomized but protocolized and instrumental variable analysis was used to obtain comparable groups, and that a limited spread of ethnicities was included.

Conclusions

Although the disease activity has improved in both autoantibody-positive and autoantibody-negative RA in recent decades, the response in long-term outcomes differed. We propose that it is time to subdivide RA in autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2), in the hope that this leads to stratified treatment in RA.

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AUTHOR SUMMARY

Why Was This Study Done?

- Patients with rheumatoid arthritis (RA) have different risk factors and histology (microscopic anatomy) depending on the presence or absence of autoantibodies (anti-citrullinated protein antibodies and rheumatoid factor).
- Because it is suspected that RA with and without autoantibodies are two distinct diseases with a different pathophysiology, we hypothesized that these two types of RA react differently to improvements in treatment strategies that have taken place over the last decades.

What Did the Researchers Do and Find?

- Since its start in 1993, the inclusion criteria of the Leiden early arthritis cohort have not changed and included RA patients remained similar, apart from earlier diagnosis, therefore RA patients from different years were comparable. Treatment protocols enhanced over time, but were similar for patients with and without autoantibodies.
- We studied the changes in disease activity and three long term outcomes of RA patients with and without autoantibodies over time (inclusion period was a proxy for treatment strategy).
- We found that while disease activity improved in both patient groups, the long term outcomes (the possibility to permanently stop medication, mortality and functional disability) only improved in RA patients with autoantibodies.

What Do These Findings Mean?

- The disconnection between improvement in disease activity and subsequent improvement in longterm outcomes in RA without autoantibodies suggest that the underlying pathogenesis of RA with and without autoantibodies is different.
- We propose that it is time to formally subdivide RA into type 1 (with autoantibodies) and type 2 (without autoantibodies).

INTRODUCTION

Careful clinical observations over time have led to the description of diseases. In addition, subdividing of diseases has also been based on clinical observations, whilst differences in pathogenetic aetiology were identified subsequently. For instance subdividing diabetes in type 1 and type 2 was based on differences in clinical presentation (young versus older and obese patients); this distinction was confirmed by treatment response to insulin, and subsequently fuelled targeted etiological studies [1].

Rheumatoid arthritis (RA) is considered a syndrome. During the last decade it was observed that there are differences in RA-patients with and without autoantibodies (such as Rheumatoid factor (RF) and anti–citrullinated protein antibodies (ACPA)). Autoantibody-positive RA has a different genetic background [2], different environmental risk factors [3,4], slight differences in the preclinical symptomatic phase and first clinical presentation [5-7], differences in histology [8], differences in the synovial fluid cytokine profile [9] and, when left untreated, more severe joint destruction [5]. Nonetheless, the aetiology and pathophysiology of RA is still incompletely understood. It is unclear if there is one pathophysiological genesis, in which the presence of autoantibodies is promoted by certain genetic factors and where autoantibodies act as a 'severity' factor. Or, alternatively, that there are two different mechanisms of disease development. When distinct disease-mechanisms exist, treatment response may differ. Whether autoantibody-positive and autoantibody-negative RA have different mechanisms can therefore be addressed by clinical evaluation of long-term results in response to changes in treatment strategy.

Slight differences in effect of some drugs have been described between autoantibody-positive and autoantibody-negative RA-patients based on trial-data [10-13], but these are based on selected groups of RA-patients with a limited follow-up duration. We will take advantage of a large longitudinal cohort including incident RA-patients without selection from a region during the last 25 years; to our knowledge this is currently the largest observational cohort of RA. Treatment of RA has changed over time and improvements in strategies (e.g. early start, treat-to-target treatment adjustments) were not different for autoantibody-positive and autoantibody-negative patients. To evaluate whether autoantibody-positive RA and autoantibody-negative RA are two disease types, we studied the associations between changing treatment-strategies and disease activity in the short-term as well as three long-term outcomes.

METHODS

Longitudinal cohort

The Leiden Early Arthritis Clinic is a population based inception cohort including all consecutive patients newly presenting with recent-onset arthritis, that was started in 1993 and has been described in [14]. Inclusion criteria were presence of synovitis determined at physical examination by rheumatologists and symptom duration of <2 years. The department of rheumatology in the Leiden University Medical Center is the only centre for rheumatic diseases in a semi-rural area with >400,000 inhabitants. Since the start of the cohort general practitioners (GPs) were informed on the relevance of early referral and patients referred with suspicion on early arthritis were seen with priority, generally <2 weeks. Of note, in line with Dutch GP-guidelines, autoantibodies were rarely determined in primary care [15]. Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee ('Commissie Medische Ethiek' of the Leiden University Medical Centre; B19.008).

For this study we selected the patients with RA (clinical diagnosis plus fulfilment of 1987-ACR-criteria). The use of the 1987-criteria (instead of the 2010-criteria) excluded influences of temporary changes in views on diagnosing RA and of the inverse relationship between presence of autoantibodies and degree of inflammation on the classification [16,17]. Between 2/24/1993 and 31/12/2016, 1377 patients enrolled in the cohort were classified with RA.

At the first visit, rheumatologists and patients completed questionnaires (among which the health assessment questionnaire disability index (HAQ)), swollen and tender joint counts (SJC, TJC) were performed, and blood samples taken for routine diagnostic laboratory screening (including erythrocyte sedimentation rate (ESR), immunoglobulin M– rheumatoid factor (positive if ≥3.5 IU/ml). From 2006, ACPA (anti-CCP2, Eurodiagnostica, positive if ≥25 U/ml; from 2009 EliA CCP, Phadia, positive if ≥7U/ ml) was measured. In patients included before 2006, ACPA-status was assessed retrospectively on stored baseline serum samples using the Eurodiagnostica assay. Since seroconversion is rare, repeated ACPA and/or RF measurements during follow-up were not studied [18]. In six patients autoantibody-status was not available, consequently they were excluded from the analyses (S1 Fig).

Protocolized follow-up visits were performed twice in the first year and yearly thereafter, as long as patients were treated at the outpatient clinic. Follow-up ended in case of death, release from care due to sustained DMARD-free remission (SDFR), moving to another area or withdrawal of informed consent while remaining treated. As data were collected at regular rheumatologist visits withdrawal of informed consent was rare. Data from the Statistics Netherlands from our region showed that moving away from the Leiden area was also infrequent (<3% annually) [19]. Inherent to the design, follow-up was shorter in the more recent inclusion periods. The majority of missing follow-up visits (not due to inclusion date) was due to mortality or SDFR.

Definition autoantibody-positive and autoantibody-negative

Patients with ACPA and/or RF were categorized as autoantibody-positive; double negative patients as autoantibody-negative. For practical reasons the distinction in type 1 and type 2 respectively is based on the autoantibodies that are currently used in the clinic. It could be that if more factors were included, eg other autoantibodies or other factors such as obtained from histology, a better division into groups would have been obtained [20-23]. Our primary goal, however, was to investigate the main distinction into autoantibody-positive and autoantibody-negative RA as it is used in clinical practice.

Treatment

Patients were treated in routine care according to protocols. 86 of 1377 RA-patients were treated within randomized clinical trials that were not in line with the treatment guidelines at that time and excluded, leaving 1285 RA-patients for analyses (S1 Fig). Temporal changes in treatment strategies concerned the initial start as well as treatment adjustments over time; both improvements in strategies are reflected by inclusion period as proxy. Patients included between 2/24/1993-31/12/1996 (n=168) received initial NSAIDs and started mild DMARDs with delay. Patients included between 1/1/1997-31/12/2000 (n=185) were treated early but not with methotrexate (e.g. hydroxychloroquine and sulfasalazine) [24]. Patients included between 1/1/2001-31/12/2005 (n=207) started early with methotrexate [25]. From 2006 onwards early methotrexate was followed by treat-to-target treatment adjustments, indicating treatment adjustments in case of increase disease activity scores (DAS) (1/1/2006-31/12/2010, n=335) [26]. Furthermore, because the value of very early treatment became even more apparent in 2010, and as GP-delay contributed most to the total delay in our region [27], from 2011 onwards on top of the existing regimen additional efforts were undertaken to further reduce referral delay by instituting an early arthritis recognition clinic, which is a screening clinic for the presence of inflammatory arthritis (1/1/2011-31/12/2016, n=390) [27-29].

In line with absence of guidelines that initial treatment should be adapted to autoantibody status [30,31], initial treatment choices were not directed by autoantibodies. Subsequent treatment decision were targeted at DAS; this was independent of patient characteristics. Thus protocols were similar for type 1 and 2.

Anti-TNF was the first biologic that became available in the early 2000s for RA-patients that failed on ≥2 conventional DMARDs [32]. Over time other biologics were registered, though the indication remained similar in the Netherlands. S1 Table provides information about the use of biologics at different follow-up durations, for type 1 and 2 separately. The usage was slightly higher in type 1, especially after introduction of treat-to-target.

Outcomes

Disease activity reflected the direct results of treatment; measured with the DAS28-ESR [33]. Since 2006 treatment is aimed at this short-term target to eventually improve long-term outcomes. Three long-term outcomes were studied: SDFR, mortality and functional disability. SDFR was defined as the sustained absence of synovitis (by physical examination) after discontinuation of DMARD therapy (including biologics, systemic or intra-articular corticosteroids) for the entire follow-up after DMARD-withdrawal, and this follow-up had to be at least one year after DMARD-stop [34]. This stringent and innovative definition of long-term remission is the opposite of disease persistence and became increasingly achievable [35]. After achievement of SDFR, patients were followed for median 5.5 years, to verify its sustainability. Patients that achieved DMARD-free remission but developed a late flare during this follow-up (n=23) were not considered as being in SDFR. All medical files of patients with ≥1 year follow-up were retrospectively explored on SDFR until April 2017. Mortality status was obtained from the civic registries on June 1, 2018. Functional disability, is one of the most important outcome from patients' perspective [36], and was measured yearly with the HAQ ranging from 0-3 (no-severe disability) [37,38].

Statistical Analyses

Main analyses were done for type 1 and 2 RA separately. Inclusion period was used as instrumental variable for treatment strategy. Within each type, improvements over time were compared to the reference period (inclusion 1993-1996).

Next, improvements over time compared to the reference period were compared between the two types by including an interaction term in the models to quantify the difference in improvent over time between the two types.

Time to SDFR was analysed with Cox regression. SDFR-status was censored at the date of revision of the medical files or at an earlier date when they were lost to follow-up or had died.

Mortality was analysed with Cox regression; follow-up was censored at the date of data extraction. Mortality was not compared to the general population because determination of excess mortality in RA relative to the population requires >10 years of follow-up to become apparent [39,40]; this follow-up duration was absent for the recent inclusion periods.

Missing data on DAS (complete DAS missing, 0% baseline and 3% follow-up) and HAQ (13% baseline, 22% annual follow-up) of attended visits were imputed using multivariate multiple imputation with predictive mean matching (100 cycles, 30 datasets). DAS and HAQ were analysed with linear mixed models. Because both outcomes rapidly decreased within the first year, the first year was analyzed separately from the remaining follow-up [41-43]. Slope of decrease in the first year was analysed with a random intercept and an identity covariance matrix. The course after the first year was analysed with a random intercept, random slope and continuous auto-regressive covariance matrix of order 1. Estimated marginal means were calculated. Percentages of DAS28-ESR remission (<2.6) at 1 and 3 years were tested with chi-square tests [44].

To minimize the influence of the association of the studied exposure and follow-up duration, analyses were truncated at 15 years follow-up and follow-up duration was not included as covariate in any of the analyses. All analyses were corrected for age and gender to improve model fit. As none of the measured baseline covariates are true confounders on the relationship between treatment strategy and outcomes, because they are not associated with the exposure or regarded to be the causal path (see S1 Text and S2 Fig for explanation), no other corrections were made.

No formal prospective analysis plan was written down and submitted prior to performing the analyses. Widths of the intervals have not been adjusted for multiplicity and p-values <0.05 were considered significant. R 3.6.1 with packages described in Text S2 were used. This study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (See S1 Checklist).

Sensitivity Analyses

In a sensitivity analysis RA was defined according to the 2010-criteria.

In response to requests during peer review, to assess whether the difference in age at onset between the disease types might influence the results, patients aged <65 years at diagnosis were analysed in a sensitivity analysis.

For SDFR and mortality a sensitivity analysis was done, as due to differences in symptom duration at baseline, patients could not have presented themselves to the EAC because the studied event (SDFR, death) had already happened. To assess the influence of this possible left-truncation, correction for left-truncation was applied.

Finally, data for both disease types were plotted per inclusion periods for all outcomes; this was done for illustration.

RESULTS

Baseline characteristics

823 patients had type 1 RA; the mean age at first presentation was 55, 67% was female (Table 1). 462 patients had type 2; their mean age was 60, 64% was female. Age, gender and percentage of RA types were constant throughout the inclusion periods (p=0.59, p=0.28 and p=0.42, respectively), showing that similar RA-patients were included over time. Within both RA types, patients presented with shorter symptom duration, lower numbers of swollen and tender joints and lower acute phase reactants in more recent inclusion periods, reflecting that earlier presentation was paralleled with less severe disease (Table 1).

Disease activity

In type 1 RA, DAS improved in the first year and during subsequent follow-up (Fig 1; Table 2). Percentage of patients achieving DAS28-ESR remission (<2.6) significantly increased, e.g. from 13% in the oldest inclusion period, to 50% at year 1 and 61% at year 3 in the most recent period (S3 Fig).

In the type 2 RA, DAS also improved, especially in the first year (Fig 2; Table 3). DAS28-ESR remission percentages increased from 32% in the oldest inclusion period, to 54% at year 1 and 71% at year 3 in the most recent period (S3 Fig).

Sustained DMARD-free remission

In type 1 RA, SDFR significantly increased over time, especially since the start of treat-to-target (Fig 1; Table 2). In type 2 RA, there was no significant increase in SDFR (Fig 2; Table 3).

Mortality

Compared to the reference period, mortality decreased significantly in type 1 RA since the start of treat-to-target (Fig 1; Table 2). No significant association was found in type 2 RA (Fig 2; Table 3), although hazard ratios were in the same direction as in type 1 RA.

Functional Disability

In type 1 RA, functional disability improved over time since the start of early methotrexate, both in the first year and the subsequent years (Fig 1; Table 2). In type 2 in contrast, improvement was absent (Fig 2; Table 3).

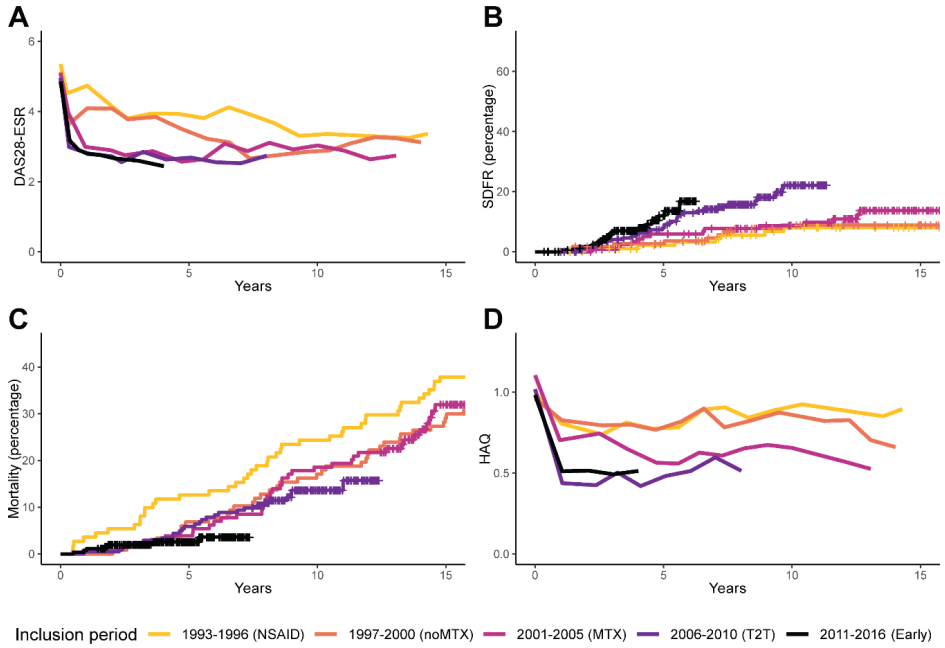
Table 1: Characteristics of patients with type 1 (autoantibody-positive; A) and type 2 RA (autoantibody-negative; B) at first presentation to the early arthritis clinic.

	1993-1996 (n = 112, 67%)	1997-2000 (n=118, 64%)	2001-2005 (n = 129, 62%)	2006-2010 (n = 203, 61%)	2011-2016 (n = 261, 67%)	p-value
Women, n (%)	77 (69)	82 (70)	91 (71)	136 (67)	167 (64)	0.70
Age in years, mean (SD)	56 (16)	55 (16)	55 (15)	54 (15)	56 (15)	0.63
Symptom duration, days median (IQR)	153 (84-306)	156 (84-304)	147 (72-264)	146 (61-270)	103 (53-227)	0.006
Current smoker, n (%)	35 (33)	35 (33)	29 (27)	40 (22)	74 (30)	0.21
28-SJC, median (IQR)	6 (3-10)	7 (4-12)	4 (2-7)	4 (2-7)	4 (2-7)	<0.001
28-TJC, median (IQR)	7 (3-13)	7 (3-14)	7 (3-12)	6 (3-11)	5 (2-9)	<0.001
ESR, median (IQR)	46 (26-70)	32 (20-54)	30 (18-55)	29 (14-42)	29 (14-41)	<0.001
VAS general health, median (IQR)	43 (17-70)	44 (26-66)	53 (34-72)	56 (29-72)	70 (50-80)	<0.001
DAS28-ESR, median (IQR)	5.5 (4.2-6.5)	5.2 (4.2-6.1)	5.2 (4.3-6.0)	4.9 (4.2-6.0)	4.8 (4.1-5.7)	0.02
HAQ, median (IQR)	1.0 (0.6-1.4)	0.8 (0.4-1.6)	1.0 (0.6-1.6)	1.0 (0.5-1.5)	1.0 (0.5-1.5)	0.12

B.	1993-1996 (n = 56, 33%)	1997-2000 (n = 67, 36%)	2001-2005 (n = 78, 38%)	2006-2010 (n = 132, 39%)	2011-2016 (n = 129, 33%)	p-value
Women, n (%)	38 (68)	41 (61)	57 (73)	80 (61)	79 (61)	0.34
Age in years, mean (SD)	56 (15)	59 (19)	60 (14)	61 (16)	62 (14)	0.16
Symptom duration, days median (IQR)	126 (61-220)	92 (62-219)	120 (74-234)	109 (59-176)	85 (45-189)	0.06
Current smoker, n (%)	17 (30)	11 (18)	14 (20)	24 (21)	28 (22)	0.52
28-SJC, median (IQR)	9 (4-14)	12 (7-19)	6 (3-10)	6 (3-10)	6 (3-10)	<0.001
28-TJC, median (IQR)	9 (3-19)	13 (6-20)	11 (5-19)	9 (4-13)	7 (3-11)	<0.001
ESR, median (IQR)	40 (22-56)	28 (16-47)	27 (16-47)	31 (9-46)	25 (11-41)	0.008
VAS general health, median (IQR)	46 (25-63)	50 (26-62)	56 (36-75)	64 (44-79)	70 (60-80)	<0.001
DAS28-ESR, median (IQR)	5.6 (4.5-6.3)	5.8 (4.8-6.5)	5.6 (4.4-6.7)	5.3 (4.4-6.3)	5.2 (4.4-6.0)	0.19
HAQ, median (IQR)	1.1 (0.8-1.6)	0.9 (0.5-1.4)	1.1 (0.8-1.8)	1.1 (0.8-1.5)	1.0 (0.6-1.5)	0.15

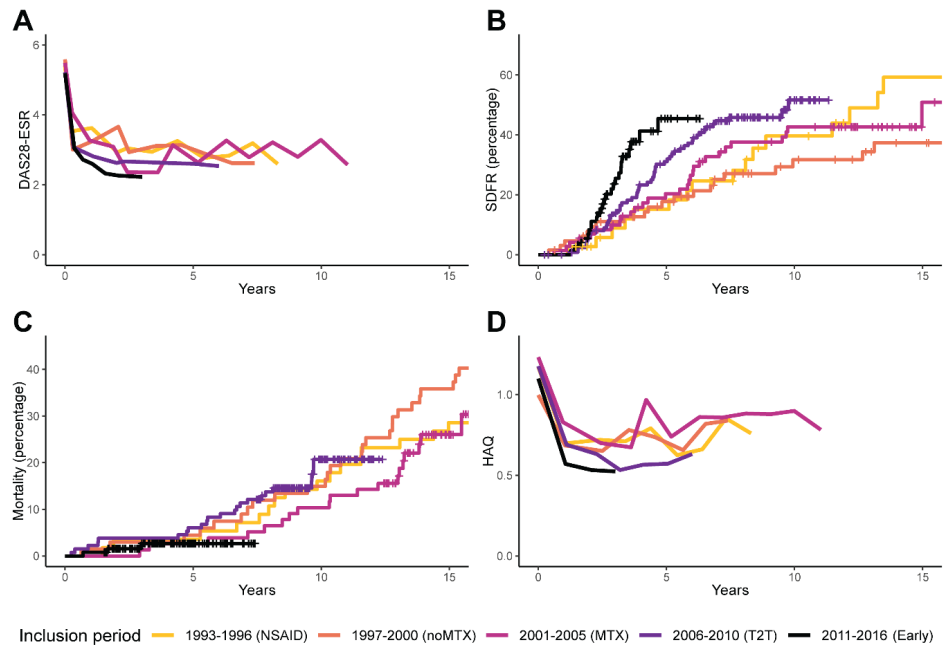
Legend: N, number of patients; SD, standard deviation; IQR, inter quartile range; SJC, swollen joint count; TJC, tender joint count; ESR, erythrocyte sedimentation rate; VAS, visual analogue scale; DAS, disease activity score; HAQ, health assessment questionnaire. p-value; results of Kruskal-Wallis H-test (Fisher's exact test for proportions and ANOVA for normally distributed variables).
The percentage of patients with type 1 or 2 RA for the different inclusion periods was stable over time (p=0.42).
The 28-SJC and 28-TJC counts are the number of swollen and tender joints, respectively, out of 28 joints assessed.
The VAS general health is a self-reported assessment, ranging from 0 to 100.
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity.
The HAQ (HAQ-DI) ranges 0-3, with higher scores indicating more disability.

Figure 1: Disease activity over time (A) and the long-term outcomes sustained DMARD-free remission (B), mortality (C) and functional disability (D) in type 1 (autoantibody-positive) RA.



Legend: For DAS28-ESR and HAQ, mean values of imputed data from visits that were attended are shown; when <20% of patients attended the visit, lines were truncated.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; SDFR, sustained DMARD-free remission; HAQ, health assessment questionnaire; NSAID, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; T2T, treat-to-target; Early, early treatment;
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity. Remission is defined as a score <2.6 and a change of >1.2 is considered a clinically relevant change [44].
The HAQ ranges 0-3, with higher scores indicating more disability. The minimally important difference is 0.22 [38].
For SDFR, at 5 years, 85%, 87%, 89%, 82% and 32% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 79%, 71%, 70%, 15%, 0% and at 15 years 56%, 59%, 12%, 0%, 0%.
For mortality, at 5 years, 87%, 93%, 96%, 94% and 42% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 76%, 83%, 81%, 38%, 0% and at 15 years 62%, 71%, 35%, 0%, 0%.

Figure 2: Disease activity over time (A) and the long-term outcomes: sustained DMARD-free remission (B), mortality (C) and functional disability (D) in type 2 (autoantibody-negative) RA.



Legend: For DAS28-ESR and HAQ, mean values of imputed data from visits that were attended are shown; when <20% of patients attended the visit, lines were truncated.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; SDFR, sustained DMARD-free remission; HAQ, health assessment questionnaire; NSAID, nonsteroidal anti-inflammatory drugs; MTX, methotrexate; T2T, treat-to-target; Early, early treatment.
The DAS28-ESR ranges 2-9.4, with higher scores indicating more disease activity. Remission is defined as a score <2.6 and a chnge of >1.2 is considered a clinically relevant change [44].
The HAQ ranges 0-3, with higher scores indicating more disability. The minimally important difference is 0.22 [38].
For SDFR, at 5 years, 73%, 74%, 72%, 62% and 14% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 41%, 45%, 47%, 9%, 0% and at 15 years 22%, 31%, 8%, 0%, 0%.
For mortality, at 5 years, 96%, 96%, 97%, 94% and 27% of patients from inclusion period 1993-1996 to 2011-2016, respectively, were still in at risk. At 10 years, this was 84%, 85%, 90%, 34%, 0% and at 15 years 71%, 64%, 26%, 0%, 0%.

Table 2: Disease activity during the first year and subsequent follow-up and long-term outcomes: sustained DMARD-free remission, mortality and functional disability per inclusion period compared to the reference period for type 1 (autoantibody-positive) RA.

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	-0.38 (-0.87;0.10)	0.12	**-0.41 (-0.66;-0.16)**	**0.002**	1.14 (0.42;3.05)	0.80	0.74 (0.47;1.15)	0.18	0.01 (-0.19;0.21)	0.89	-0.02 (-0.15;0.11)	0.58
2001-2005	**-1.70 (-2.21;-1.20)**	**<0.001**	**-0.86 (-1.12;-0.61)**	**<0.001**	1.66 (0.67;4.12)	0.27	0.71 (0.46;1.11)	0.13	**-0.28 (-0.49;-0.07)**	**0.009**	**-0.16 (-0.29;-0.03)**	**0.043**
2006-2010	**-1.62 (-2.08;-1.17)**	**<0.001**	**-1.04 (-1.28;-0.80)**	**<0.001**	**3.35 (1.46;7.72)**	**0.004**	**0.56 (0.34;0.92)**	**0.023**	**-0.33 (-0.51;-0.14)**	**0.001**	**-0.32 (-0.44;-0.20)**	**<0.001**
2011-2016	**-1.54 (-1.96;-1.12)**	**<0.001**	**-1.07 (-1.32;-0.83)**	**<0.001**	**4.57 (1.80;11.6)**	**0.001**	**0.33 (0.14;0.77)**	**0.010**	**-0.29 (-0.46;-0.12)**	**0.001**	**-0.26 (-0.38;-0.14)**	**0.008**

Bold numbers indicate p-values < 0.05.
[a] Difference in slope in the first year compared to the slope in 1993-1993; analyzed with linear mixed models corrected for age and gender. A negative number indicates a steeper slope.
[b] Difference in mean over time compared the mean over time in 1993-1996; analyzed with linear mixed models corrected for age and gender.
[c] Hazard ratios compared to 1993-1996; analyzed with Cox regression corrected for age and gender.
[d] The estimated marginal mean, adjusted for age and gender, in type 1 RA for inclusion period 1993-1996 was -0.34 (-0.70 to 0.03) for the slope in DAS28-ESR in the first year, 3.58 (3.39 to 3.76) for DAS28-ESR over time after the first year, -0.15 (-0.29 to 0.00) for slope in HAQ in the first year and 0.78 (0.68 to 0.88) for HAQ over time after the first year.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; p-val, p-value.

Table 3: Disease activity during the first year and subsequent follow-up and the long-term outcomes: sustained DMARD-free remission, mortality and functional disability per inclusion period compared to the reference period for type 2 (autoantibody-negative) RA.

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	-0.53 (-1.30;0.24)	0.18	0.08 (-0.32;0.49)	0.69	0.61 (0.32;1.18)	0.14	0.67 (0.35;1.30)	0.24	0.16 (-0.13;0.44)	0.29	0.03 (-0.19;0.24)	0.81
2001-2005	**-0.88 (-1.66;-0.11)**	**0.025**	-0.03 (-0.43;0.37)	0.89	0.80 (0.43;1.48)	0.48	0.57 (0.28;1.13)	0.11	0.05 (-0.25;0.35)	0.75	0.10 (-0.12;0.31)	0.38
2006-2010	**-0.78 (-1.48;-0.08)**	**0.029**	-0.26 (-0.63;0.11)	0.17	1.11 (0.63;1.97)	0.71	0.79 (0.40;1.56)	0.50	0.02 (-0.24;0.28)	0.87	-0.09 (-0.28;0.10)	0.34
2011-2016	**-1.08 (-1.75;-0.41)**	**0.002**	**-0.44 (-0.84;-0.04)**	**0.030**	1.89 (0.97;3.67)	0.060	0.36 (0.10;1.34)	0.13	-0.02 (-0.27;0.23)	0.89	-0.13 (-0.34;0.07)	0.20

Bold numbers indicate p-values < 0.05.
[a] Difference in slope in the first year compared to the slope in 1993-1993; analyzed with linear mixed models corrected for age and gender. A negative number indicates a steeper slope.
[b] Difference in mean over time compared the mean over time in 1993-1996; analyzed with linear mixed models corrected for age and gender.
[c] Hazard ratios compared to 1993-1996; analysed with Cox regression and corrected for age and gender.
[d] The estimated marginal mean, adjusted for age and gender, in type 2 RA for inclusion period 1993-1996 was -1.27 (-1.81 to -0.72) for the slope in DAS28-ESR in the first year, 2.70 (2.40 to 3.01) for DAS28-ESR over time after the first year, -0.46 (-0.67 to -0.25) for slope in HAQ in the first year and 0.62 (0.47 to 0.78) for HAQ over time after the first year. DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; p-val, p-value

Comparison of improvement of type 1 and type 2

To assess whether more improvement was indeed observed in type 1 RA compared to type 2 RA, change with respect to the reference period was compared between the two disease types by adding an interaction term to the models. More improvement for the outcomes DAS over time, SDFR and functional disability was observed in type 1 RA (Table 4). This was statistically significant for these outcomes in the inclusion period 2006-2010 (early methotrexate followed by treat-to-target treatment adjustments).

Sensitivity analyses

According to the 2010-criteria, 1421 patients had RA, 957 type 1 and 474 type 2 (S4 Fig). Due to the composition of these criteria, type 2 RA required ≥11 involved joints for classification [16,17]. Indeed this group had high joint counts, especially high tender joints in the latest periods when acute phase reactants and swollen joint counts at diagnosis decreased (S2 Table). This possibly resulted in incomparability in disease activity between the periods within type 2 RA. Results for type 1 were similar when RA was defined according to the 1987-criteria. For type 2 little improvement in DAS was present and effect sizes of long-term outcomes were in line with the main results (S3,4 Table).

Analyses were repeated in patients aged <65 years at diagnosis; similar results were obtained except for a non-significant improvement in mortality in type 1 RA, possibly caused by a lower number of events (S5,6 Table).

Effect sizes for the outcomes SFDR and mortality after correction for left truncation were similar (S7 Table).

For illustration, head-to-head comparisons between type 1 and type 2 RA within the inclusion periods are shown in S5-8 Fig.

Table 4: Differences in improvement of disease outcomes between type 1 (autoantibody-positive) and type 2 (autoantibody-negative) rheumatoid arthritis with enhanced treatment strategies over 25 years

	DAS28-ESR, slope in first year		**DAS28-ESR over time after first year**		**Sustained DMARD free remission**		**Mortality**		**HAQ, slope in first year**		**HAQ over time, after first year**	
	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val	Hazard ratio[c]	p-val	Hazard ratio[c]	p-val	Relative mean difference[a]	p-val	Relative mean difference[b]	p-val
Inclusion period												
1993-1996	Ref[d]		Ref[d]		Ref		Ref		Ref[d]		Ref[d]	
1997-2000	0.14 (-0.75;1.04)	0.75	-0.46 (-0.94;0.03)	0.068	1.80 (0.55;5.92)	0.33	1.02 (0.47;2.23)	0.96	-0.14 (-0.49;0.21)	0.42	-0.06 (-0.30;0.19)	0.65
2001-2005	-0.82 (-1.73;0.08)	0.073	**-0.70 (-1.18;-0.22)**	**0.004**	2.10 (0.70;6.28)	0.18	1.22 (0.54;2.73)	0.64	-0.33 (-0.69;0.03)	0.069	-0.21 (-0.46;0.04)	0.095
2006-2010	-0.82 (-1.64;0.00)	0.050	**-0.70 (-1.14;-0.25)**	**0.002**	**2.93 (1.08;7.90)**	**0.034**	0.82 (0.37;1.83)	0.63	**-0.35 (-0.66;-0.05)**	**0.024**	**-0.22 (-0.44;0.00)**	**0.046**
2011-2016	-0.47 (-1.23;0.29)	0.22	**-0.55 (-1.04;-0.05)**	**0.030**	2.10 (0.71;6.22)	0.18	1.11 (0.26;4.85)	0.89	-0.27 (-0.56;0.02)	0.064	-0.11 (-0.35;0.13)	0.37

Bold numbers indicate p-values < 0.05.
The overall p-value of the interaction term in the models (e.g. the p-value for difference in improvement between the two subtypes over all inclusion periods) was 0.072 for DAS28-ESR slope in first year, <0.001 for DAS28-ESR over time after first year, 0.28 for Sustained DMARD free remission, 0.91 for mortality, 0.016 for HAQ slope in first year and 0.10 for HAQ over time after first year.
[a] Additional improvement in type 1 with respect to type 2. A negative number corresponds to additional change downward in type 1 with respect to the reference period (e.g. more decrease in the first year with respect to the reference period). Since a lower DAS/HAQ is better, a negative number indicates more improvement in type 1.
[b] Additional improvement in type 1 with respect to type 2. A negative number corresponds to additional change downward of the mean after the first year in type 1 with respect to the reference period. Since a lower DAS/HAQ is better, a negative number indicates more improvement in type 1.
[c] Additional improvement in type 1 with respect to type 2. A number above 1 corresponds to additional SDFR in type 1 with respect to the reference period. Since more SDFR is better, a number above 1 indicates more improvement in type 1.
[d]Additional improvement in type 1 with respect to type 2. A number below 1 corresponds to less mortality in type 1 with respect to the reference period. Since a lower mortality is better, a number below 1 indicates more improvement in type 1.
DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire p-val, p-value.

DISCUSSION

Summary of findings

During the last 25 years, the treatment of RA has changed in several aspects. We studied outcomes of RA and observed that improved treatment strategies were paralleled by reduced disease activity in autoantibody-positive and autoantibody-negative RA, but resulting significant improvements in the long-term outcomes, SDFR, mortality and functional disability, were only present in autoantibody-positive RA and not in autoantibody-negative RA. In line with these findings, DAS, SDFR and functionality had greater improvements over the last 25 years within autoantibody-positive than within autoantibody-negative RA. Especially the introduction of treat-to-target treatment adjustments associated with significantly greater improvements in autoantibody-positive RA than in autoantibody-negative RA. The disconnection between improvements in disease activity and in several longterm outcomes suggest that the underlying pathogenesis of autoantibody-positive and autoantibody-negative RA is different. We therefore propose that the time has come to subdivide RA in type 1 and type 2.

Comparisons with other studies

Subdivisions of disease are ideally underpinned with identified differences in etiopathology. However clinical observations have frequently been the basis of subdivisions of diseases and preceded the identification of pathophysiological mechanisms. Both types of RA have a different genetic background. Whereas >100 genetic risk factors are identified for type 1, few genetic factors have been related to type 2 RA [45]. Known environmental risk factors are associated with predominantly one of the two types [3,4]. These data, together with observed differences in histology [8], may also point towards different underlying mechanisms.

Etiopathogenetic research in the last decade has focused most on autoantibody-positive RA, but a causal relationship for the autoantibodies has not been proven. Further pathogenic research is needed for both type 1 and type 2 RA.

Strengths and limitations of this study

We have studied the autoantibodies that are daily used in clinical practice (ACPA, RF). Several new autoantibodies have recently been identified; most co-occur in patients that also harbor ACPA or RF [20-23]. Few percent of ACPA- and RF-negative patients were found positive for novel autoantibodies, leaving the so-called 'serological gap' largely unchanged. There was insufficient power to assess which autoantibodies are optimal for the characterization of type 1 RA. It is a subject for further research to determine whether the division can be optimized by incorporation of recently identified autoantibodies or other markers (e.g. obtained from histology) [46].

Autoantibody-positivity was determined with the cut-offs that are also used in daily clinical practice in our hospital. Some patients might have values just around the cut-off at baseline and therefore might change in autoantibody-positivity over time. Previous research in the EAC cohort has shown that sero-conversion towards autoantibody-negativity is rare, even when SDFR is achieved, and that seroconversion was mostly caused by fluctuations of levels around the cut-off [18]. Similarly, data from our cohort show that seroconversion from autoantibody-negativity to autoantibody-positivity is also infrequent (2% after 1-year follow-up; Fig S9). Thus autoantibody status is quite stable after diagnosis.

Type 2 patients had a clinical diagnosis of RA, fulfilled classification criteria, and lacked ACPA and RF. It has been suggested that autoantibody-negative RA is heterogeneous in nature. We find it important to formally consider autoantibody-negative RA as a separate entity, but we cannot exclude that type 2 RA consists of different subtypes. This was beyond the scope and power of this study.

To assess the response to improved treatment strategies without exposing patients to outdated and less effective treatments, historical data was used and inclusion period as instrumental variable for treatment strategy. As an alternative to randomisation, instrumental variable analysis uses a proxy (inclusion period) to create groups with comparable patients that receive different treatment strategies. Between these groups, treatment strategies can be compared without confounding by indication, under the assumption that allocation to the groups is random. Since inclusion criteria of the Leiden EAC have not changed over time, year of RA diagnosis was assumed random. Importantly, initial treatment protocols and treat-to-target protocols were similar for patients with and without autoantibodies, making the instrument similar for both patient groups.

Treatment was targeted at DAS-remission since 2006, and was never targeted at autoantibodies (notable, ACPA results became available for rheumatologists in this study from 2006 onwards). While type 2 RA had a slightly higher baseline DAS and in type 1 mean DAS over time decreased more, mean DAS and remission rates were similar or better in type 2 RA in all periods. Observed differences in long-term outcomes are therefore unlikely the result of better adherence to treat-to-target in autoantibody-positive patients. Also the finding that patients with autoantibodies more often required biologics to achieve DAS-remission (S1 Table) merely underlines the difference between both types.

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Progression of joint destruction was not studied as outcome, because the natural course of type 2 RA involves little structural damage and a lack of improvement can also be explained by the inability to measure this [5]. The long-term outcomes studied here, on the other hand, had the potential for improvement, also in patients with type 2 RA.

Mortality was studied without adjusting for mortality in the general population because excess mortality in RA is heavily dependent on follow-up duration, which differs between the inclusion cohorts [40]. Although a significant improvement in mortality was observed in type 1 RA and not in type 2 RA, effect sizes were in the same direction. Analyses of longer follow-up in larger cohorts, that also adjust for mortality in the general population are needed to determine if excess mortality reduced differently between the two groups.

In current treatment strategies SDFR is not targeted. Although innovative, this is an interesting outcome from an immunological perspective, that resembles 'cure'. Prolonged follow-up duration is required to determine the sustainability of DMARD-free remission after DMARD-cessation. An advantage of our data is that we had median 5.5 years of follow-up after DMARD-stop.

RA was defined according to the 1987-criteria (not the 2010-criteria) to exclude influences of temporal changes in rheumatologists views on diagnosing RA. Furthermore, autoantibodies load heavily in the 2010-criteria. It is known that much inflammation is needed in the absence of autoantibodies to fulfill the 2010-criteria [16,17]. Even more, in our data higher tender joint counts were needed to classify RA in recent periods, possibly resulting in incomparability in DAS within the current set of autoantibody-negative 2010-RA patients. Nonetheless, similar results in long term outcomes were found.

Future implications

Possible implications of formal subdivision of RA are execution of more focused pathogenetic studies, development of treatment protocols adapted to disease type, and performance of trials per disease type. Ultimately a better distinction leads to improved personalized care.

Conclusion

In sum, to our knowledge this is the first long-term study in a large cohort of RA-patients with data of 25 years of follow-up. Based on the demonstrated differences in long-term outcomes, and supported by previous findings on risk factors, we propose to subgroup RA in type 1 and type 2, in the hope that this leads to stratified treatment in RA.

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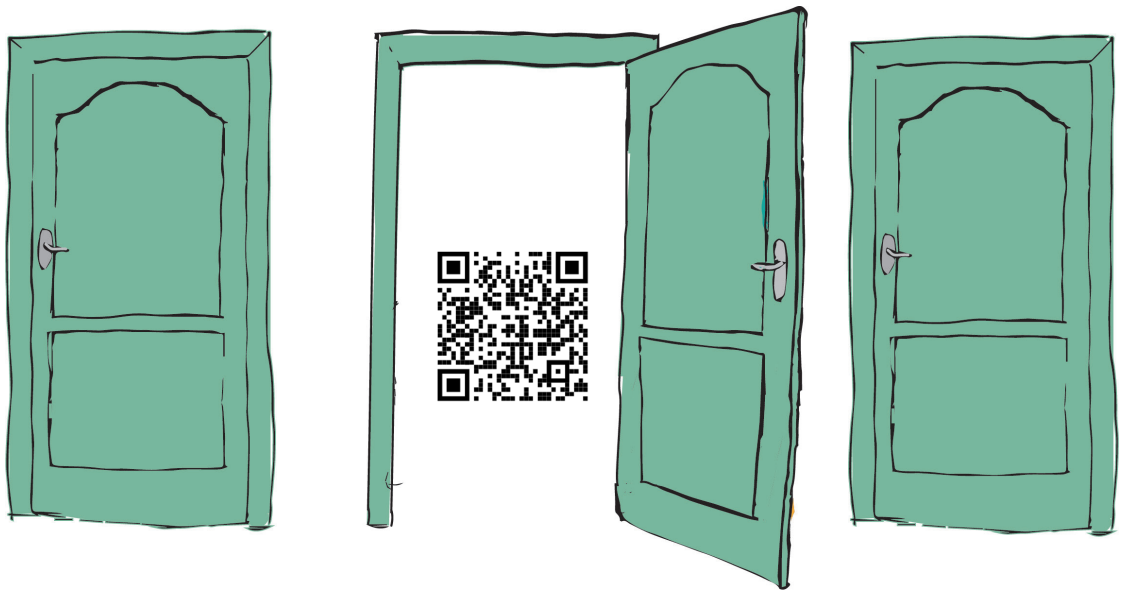

CHAPTER 8

Early intensive treatment normalizes excess mortality in ACPA-negative RA but not in ACPA-positive RA

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With great interest we read the recently published report by Poppelaars et al. in which no excess mortality was observed in 155 rheumatoid arthritis (RA) patients from the COBRA-trial, who received early intensive treatment, compared to the general population (Standardized mortality rate (SMR) 0.80 (0.59-1.06)).[1] The question whether mortality in RA has normalized is debated, as contradicting results have been published.[2-8] In many of the studies on mortality two important factors are not sufficiently taken into account: follow-up duration and disease subtypes. This might explain the conflicting results. Because thus far none of the reported studies incorporated both factors in the analyses, it is too soon to conclude that mortality is "normal" again, as we will show here.

We compliment the authors on emphasizing the importance of a long follow-up duration by showing in their meta-analysis that excess mortality in RA becomes fully apparent after >10 years. This implies that previous studies that reported on normalization of mortality had insufficient follow-up to reach this conclusion.[2-5] Some studies with a short follow-up duration even showed a seemingly decreased mortality in RA, which may be due to a healthy inclusion bias.[3-5]

RA consists of two subtypes that are characterized by the presence or absence of RA-related autoantibodies, of which the presence of anti-citrullinated protein antibodies (ACPA) is most specific for RA. Both subtypes have known differences in the severity of the disease course. The study of Poppelaars et al did not stratify for ACPA, which is due to a small sample size (n=155), leaving the question unanswered if mortality has normalized in both subsets of RA.

To assess the true impact of early intensive treatment on mortality, we performed a large study with up to 25 years of follow-up and sufficient power to stratify for ACPA. 1288 RA-patients fulfilling the 1987 criteria, who were consecutively included in the Leiden Early Arthritis Clinic, were studied. According to treatment in routine care, patients included between 1993-2000 received initial treatment with only NSAIDs or mild DMARDs (e.g. penicillamine, gold, hydroxychloroquine). Patients included between 2001-2016 were treated with early intensive treatment with methotrexate as first-line treatment. Treat-to-target became routine during this period as well. Mortality data were obtained from the civic registries on June 1, 2018. Mortality was compared to the general population in the Netherlands with SMRs adjusted for birth year, gender and calendar year. SMRs were determined for both treatment-strategies, after stratification for follow-up duration (0-5 years, 5-10 years, >10 years) and disease subset (ACPA-status).

Baseline characteristics are shown in Table 1. 248 patients died during follow-up. SMRs increased during follow-up and excess mortality became evident after 10 years of disease (0-5 years SMR 0.55 (0.41-0.73); 5-10 years 1.08 (0.87-1.33) and >10 years 1.39 (1.15-1.66); Figure 1A). Stratification for disease subset revealed that a decreased mortality was observed within ACPA-negative RA (SMR 0.80 (0.67-0.96)) and an increased mortality within ACPA-positivity RA (SMR 1.38 (1.15-1.63); Figure 1B). Comparing the two treatment strategies without considering follow-up duration and ACPA-status revealed that early intensive treatment was associated with a decrease in mortality compared to the general population (SMR 0.77 (0.63-0.93)), in contrast to group without early intensive treatment (SMR 1.23 (1.05-1.44); Figure 1C). This is concordance with the findings from Poppelaars et al. Subsequent stratification for follow-up duration and ACPA-status showed that excess mortality became apparent after 10 years of disease in ACPA-negative RA without early intensive treatment and that early intensive treatment had normalized this excess mortality. In ACPA-positive RA, in contrast, excess mortality emerged after 5 years of follow-up and was not influenced by early intensive treatment.

Table 1: Baseline characteristics of RA patients treated without and with early intensive treatment

	No early intensive treatment (n = 353)	Early intensive treatment (n = 945)
Inclusion period	1993-2000	2001-2016
Women, n (%)	238 (67)	620 (66)
Age in years, mean (SD)	56 (16)	58 (15)
Symptom duration, days median (IQR)	136 (75-279)	117 (58-234)
Current smoker, n (%)	98 (30)	211 (25)
ESR, median (IQR)	37 (21-58)	29 (14-45)
66-SJC, median (IQR)	10 (5-16)	6 (3-11)
RF-positive, n (%)	193 (55)	543 (59)
ACPA-positive, n (%)	199 (56)	456 (51)

Legend: N, number of patients; SD, standard deviation; IQR, inter quartile range; ESR, Erythrocyte sedimentation rate; SJC, swollen joint count; RF, rheumatoid factor; ACPA, anti-citrullinated peptide antibody;

In conclusion, sufficient follow-up duration and stratification for relevant disease subsets are important to disentangle the effects of treatment on mortality. Our data from a large cohort of RA patients with up to 25 years follow-up showed that excess mortality has resolved since the introduction of early intensive treatment in ACPA-negative RA, but excess mortality remains an issue in ACPA-positive RA. This underlines that RA consists of two types with differences in treatment response and long-term outcome and that additional efforts are still needed to reduce the increased risk of early death in ACPA-positive RA.

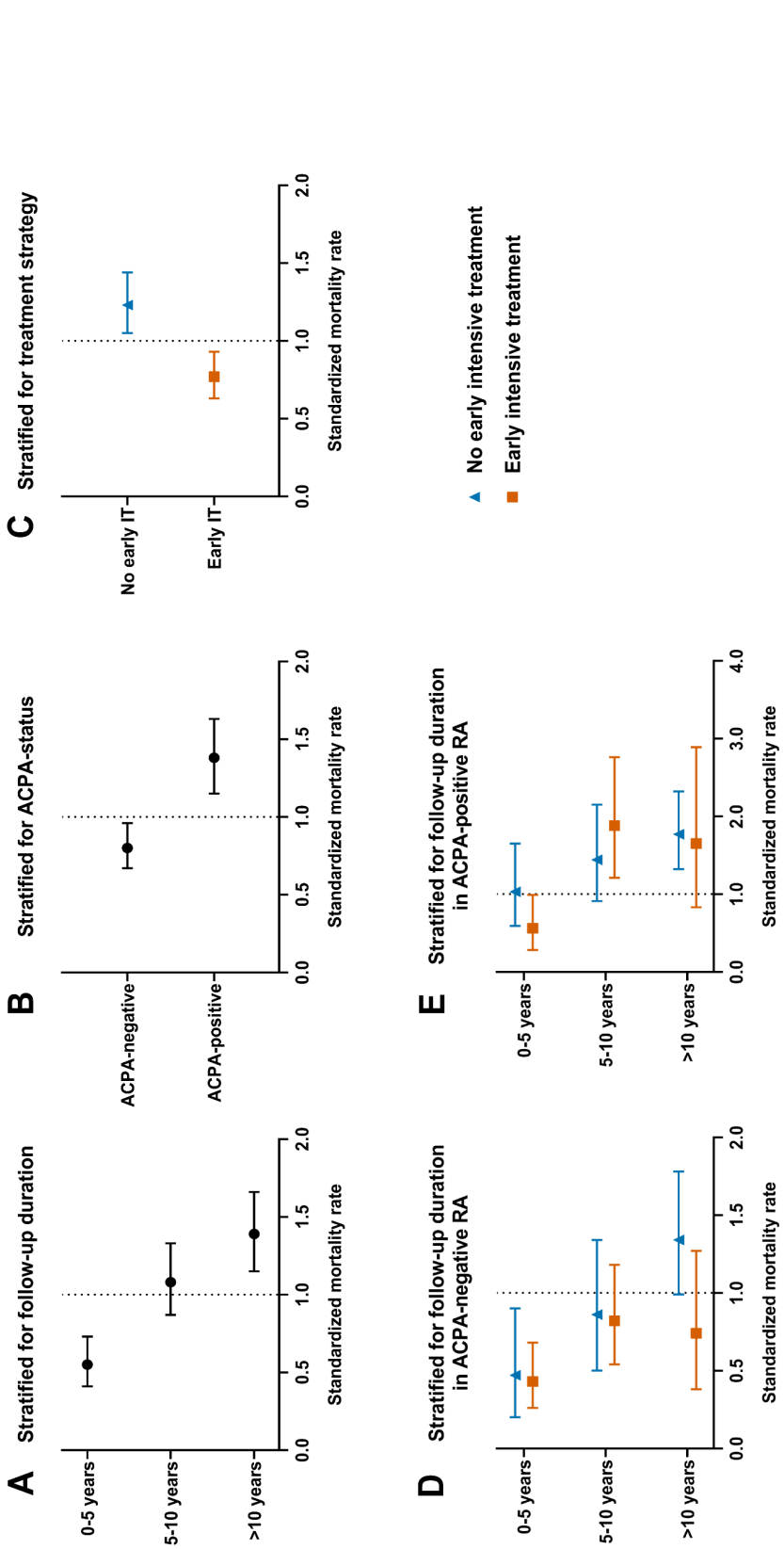

Figure 1: Mortality of patients with RA compared to the general population, stratified for follow-up duration (A), disease subset characterized by ACPA-status (B), early intensive treatment (IT) (C) and these variables combined (D&E), showing that excess mortality has normalized by early intensive treatment in ACPA-negative RA but not in ACPA-positive RA.

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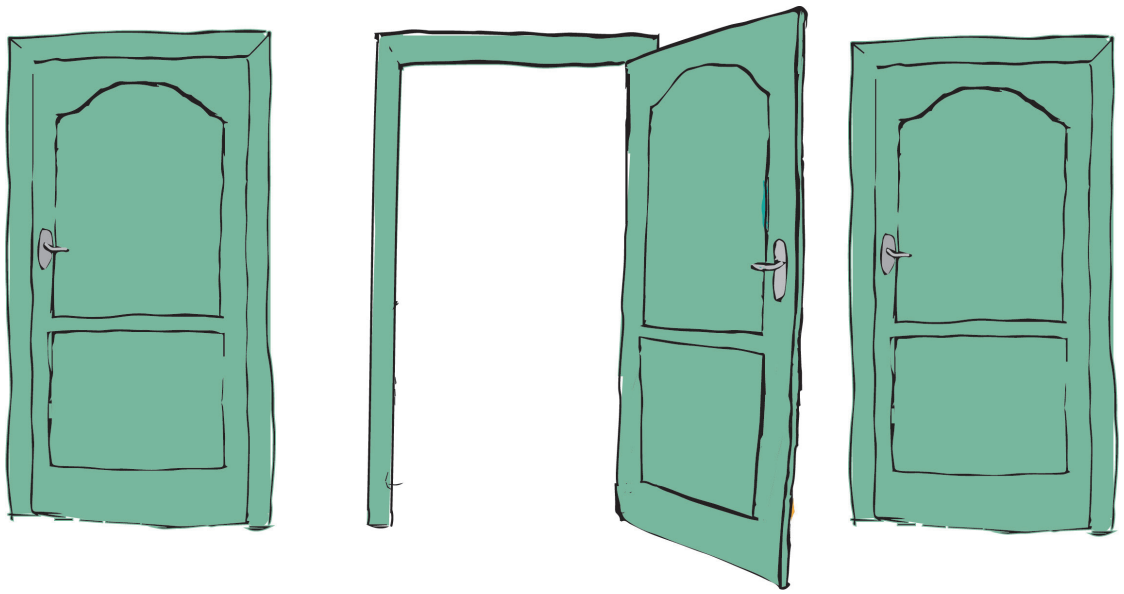
CHAPTER 9

Summary and discussion



In this thesis we aimed to assess the differences and similarities between autoantibody-positive and autoantibody-negative RA from the start of the of complaints to the end of the disease. We studied the symptomatic pre-arthritis phase, the early arthritis phase and long-term outcomes of rheumatoid arthritis patients. These phases were studied on the joint level with MRI, on the patient level with disease activity and patient reported outcomes (PROs) and on the society level using data from all rheumatoid arthritis patients from the Leiden region that presented to the LUMC since 1993.

SUMMARY OF FINDINGS

Pre-arthritis

In **Chapter 2**, we analysed which combinations of MRI-features at onset were predictive for RA-development in symptomatic patients without arthritis, to increase our comprehension of locations of RA-onset and to improve the predictive accuracy of MRI based on a unique cohort of clinically suspect arthralgia (CSA) patients. We identified that MCP extensor peritendinitis is among the tissues affected by RA already in the CSA phase. Furthermore, we improved prediction making. Based on the predictors "presence of MCP extensor peritendinitis" and "number of locations with subclinical inflammation" five risk categories were defined, of which the PPVs were up to 67% in the highest category. Thereafter these findings were validated in an independent set of patients, with PPVs up to 63%. The next step is to integrate these MRI data with other relevant biomarkers. Nonetheless, this enhanced the use of MRI in prediction of arthritis development in CSA patients.

Early arthritis

In **Chapter 3**, we hypothesized that if MRI-detectable tenosynovitis is a true RA-feature, the sensitivity for RA is high at diagnosis, in both autoantibody-positive and autoantibody-negative RA, and lower in other diseases. We showed that the large majority (>80%) of early RA patients have tenosynovitis at small hand and foot joints. This high sensitivity was present in both autoantibody-positive and autoantibody-negative RA, and was much lower in other arthritides. Furthermore, the sensitivity of tenosynovitis for RA was comparable to synovitis. These data imply that tenosynovitis, next to synovitis, is a true RA feature. This comprehension may fuel future research into the role of juxta-articular synovial inflammation in the pathogenesis of both autoantibody-positive and autoantibody-negative RA.

In **Chapter 4**, we determined trends in incidence of autoantibody-positive and autoantibody-negative RA over two decades in the Leiden region. We hypothesized that part of the incidence increase of autoantibody-negative RA is explained by aging of the population and this might lead to an increase of autoantibody-negative RA in the future. Using data from the Leiden EAC and population data from the Leiden area, we found an increasing incidence of autoantibody-negative RA that was absent in autoantibody-positive RA. Moreover, we show that the increase in autoantibody-negative RA is indeed in part explained by aging of the population. This will make autoantibody-negative RA more prevalent the coming years (estimated increase of ~11% in 20 years) and promotes the need for research in this subset of RA.

In **Chapter 5**, we studied the relationship between MRI detected inflammation and fatigue and found that MRI inflammation was not associated with simultaneous fatigue at diagnosis and during disease course in both autoantibody-positive and autoantibody-negative patients. Studying time orders, we observed that a decrease in MRI inflammation in the first year was associated with decrease in fatigue in the second year, however the standardized effect size was similar to clinical disease activity as measured by the DAS. Therefore, overall MRI inflammation did not aid in explaining fatigue not explained by the DAS. This suggests there is a ceiling effect for explaining fatigue by inflammation and supports the concept that fatigue in patients with classified RA is in part disconnected from inflammation. Consequently, the results imply that aiming at imaging remission instead of clinical remission does not lower fatigue in autoantibody-positive and autoantibody-negative RA.

In **Chapter 6**, we studied patterns of MRI inflammation decrease in 216 consecutive RA and UA patients who received early DMARD-treatment. We used cross-lagged models to evaluate the influence of two time-patterns: a simultaneous pattern ("change in one inflammatory feature associated with change in another feature") and a subsequent pattern ("change in one inflammatory feature preceded change in another feature"), in three time-periods (0-4 months, 4-12 months, 12-24 months). We observed a simultaneous decrease of synovitis, tenosynovitis and osteitis. In addition, synovitis decrease preceded tenosynovitis decrease. In autoantibody-positive but not in autoantibody-negative patients, synovitis decrease preceded osteitis decrease. Therefore patters of subsequent change were partly different in the autoantibody-positive and autoantibody-negative disease. This suggests that different inflammatory pathways underlie MRI-inflammation in autoantibody-positive and autoantibody-negative RA.

Long-term outcomes

In **Chapter 7**, we studied the response of long-term outcomes of autoantibody-positive and autoantibody-negative RA patients to treatment strategies that have changed over the last 25 years. We observed that included RA patients had remained similar, apart from earlier diagnosis; therefore, RA patients from different years were

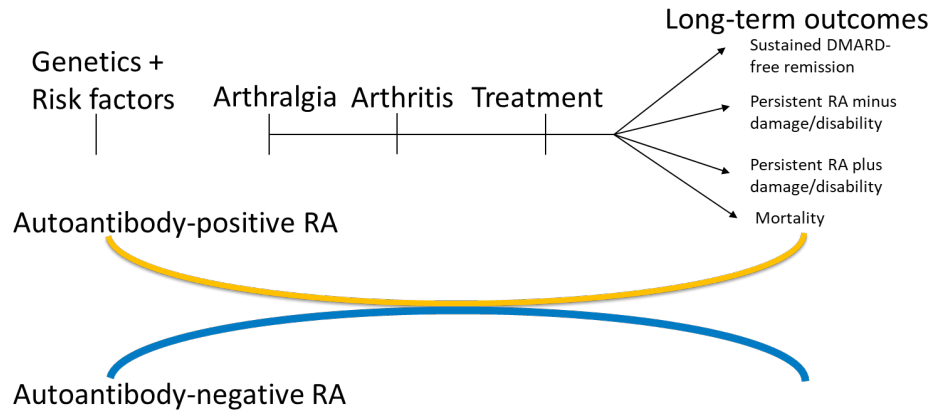
comparable. We found that while disease activity improved in both autoantibody-positive and autoantibody-negative RA patients, the long-term outcomes (the possibility to permanently stop medication, mortality, and functional disability) only improved in autoantibody-positive RA patients. The disconnection between improvement in disease activity and subsequent improvement in long-term outcomes in RA without autoantibodies suggests that the underlying pathogenesis of RA with and without autoantibodies is different. Based on our data, we think it is time to make a differentiation in RA and accordingly divide it into autoantibody-positive (type 1) and autoantibody-negative (type 2) subsets. This differentiation will stimulate focused etiopathologic studies as well as stratified clinical trials.

In **Chapter 8**, we aimed to answer the question whether mortality in rheumatoid arthritis (RA) has normalized, as contradicting results had been published. In many of the studies on mortality two important factors are not sufficiently taken into account: follow-up duration and disease subtypes (such as autoantibody-positivity). To assess the true impact of early intensive treatment on mortality we performed a large study (>1200 RA-patients) with up to 25 years of follow-up and sufficient power to stratify for follow-up duration and autoantibody status. We showed that excess mortality has resolved since the introduction of early intensive treatment in autoantibody-negative RA, but excess mortality remains an issue in autoantibody-positive RA.

COMPARISONS WITH OTHER STUDIES

As summarized above, we studied differences and similarities of autoantibody-positive and autoantibody-negative RA from start of complaints to the end of disease. We found that these RA subtypes have many differences as well as similarities. Altogether, the amount of similarity between the two RA types seems to depend on the phase of the disease that is studied. As visualized in Figure 1, the differences between autoantibody-positive and autoantibody-negative RA are most prominent before the start of complaints and in the long-term outcomes after treatment. Conversely, the two types are more similar in the phase from the start of complaints until the initial response to treatment. In total, this implicates that autoantibody-positive and autoantibody-negative RA are two distinct diseases with different pathophysiology. Next, we will further elaborate on the course of (dis-)similarity of autoantibody-positive and autoantibody-positive RA and the implications of these (dis-)similarities.

Figure 1. Summary of differences and similarities of autoantibody-positive and autoantibody-negative RA



Pre-arthritis

Pre-arthralgia

The pre-arthritis phase generally consists of an asymptomatic and a symptomatic phase. In this thesis, the pre-symptomatic phase was not studied. However, previous research showed that autoantibody-positive and autoantibody-negative RA have major differences in this phase: They have different genetic risk factors [1-3], different environmental risk factors [4,5] and per definition autoantibodies are not detected in autoantibody-negative RA while these are often present before complaints in autoantibody-positive RA.[6]

Prediction of arthritis development in arthralgia

In the phase of symptomatic pre-arthritis (Phase (D) according to the EULAR study group for risk factors for RA), previous research is predominantly aimed at predicting arthritis development in either autoantibody-positive arthralgia patients or relatives of autoantibody-positive arthralgia patients.[7-9] In these autoantibody-positive arthralgia patients, morning stiffness, C-reactive protein (CRP), the shared epitope, tenderness of the joints and imaging detected inflammation have been identified as predictors for arthritis development in multiple studies.[10-12] Particularly, inflammation around the tendons as detected by imaging was shown to be predictive in this group.[8,13]

In this thesis, we studied the Leiden clinically suspect arthralgia (CSA) cohort. To our knowledge, this is the only arthralgia cohort that also includes a significant amount of autoantibody-negative patients. Previously, it was shown that MRI-detected subclinical inflammation has a positive predictive value of ~30% in CSA patients, with a negative predictive value of ~95%.[14] In **Chapter 2**, we showed that we could improve the

positive predictive value of MRI up to 75% while keeping the high negative predictive value. This was done by also incorporating the number of locations with subclinical inflammation and the presence of inflammation around the MCP tendons. More recently, we have shown that this predictive value is independent of autoantibodies.. [15] Altogether, imaging detected inflammation, particularly in the tendon sheaths, is predictive for arthritis development in both autoantibody-positive and autoantibody-negative RA.

Regarding other predictors, in concurrence with autoantibody-positive patients, CRP, shared epitope and morning stiffness are also (borderline) associated with arthritis development in CSA.[14,16] Overall, predictors for arthritis development are rather similar for autoantibody-positive and autoantibody-negative arthralgia patients.

Disease course between arthralgia and arthritis

Differences between autoantibody-positive and autoantibody-negative patients in the disease course between arthralgia and arthritis have been scarcely studied. Burgers et al. showed that autoantibody-positive and autoantibody-negative CSA patients that eventually convert to arthritis have many similarities at symptom onset and presentation with arthralgia. The differences were a higher tender joint count and more difficulties in making a fist in autoantibody-negative patients and a longer symptom duration at presentation and shorter time to arthritis in autoantibody-positive patients. [17] Ten Brinck et al. suggested that the course of MRI inflammation was similar for autoantibody-positive and autoantibody-negative patients, but autoantibody-positive patients had more osteitis when they presented with CSA.[18]

Combining these studies, it can be concluded that while some small differences can be observed at presentation with arthralgia, the predictors of arthritis development and the disease course from CSA presentation to arthritis are rather similar, except for a shorter time to arthritis development in autoantibody-positive patients. Altogether, autoantibody-positive and autoantibody-negative patients are rather similar in this phase.

Early arthritis

At presentation with arthritis, previous research showed that autoantibody-positive and autoantibody-negative RA patients are rather similar clinically: they have similar joint distribution, similar disease activity, similar disability, similar morning stiffness and similar age and gender distribution. [19-21] Conflicting results have been reported about initial treatment response: during initial treatment DAS has been reported both to be lower and higher in autoantibody-positive and autoantibody-negative patients under randomized and protocolized treatment and therefore results are inconclusive.

[22,23] In this thesis we also identified a difference in the early arthritis phase: we showed that incidence of autoantibody-negative RA was higher in the elderly (**Chapter 4**). However, altogether autoantibody-positive and autoantibody-negative RA are rather similar clinically in the early arthritis phase.

In this thesis we also studied MRI in the phase of early arthritis and initial treatment response and also found many similarities: synovitis and tenosynovitis are equally as often present at first presentation (**Chapter 3**); MRI inflammation does not help in explaining fatigue in both autoantibody-positive and autoantibody-negative RA (**Chapter 5**); All inflammatory features decrease simultaneously after initial treatment and synovitis decrease precedes tenosynovitis decrease (**Chapter 6**). We also identified one difference: in autoantibody-positive but not in autoantibody-negative patients, synovitis decrease preceded osteitis decrease in the second year (**Chapter 6**). Altogether, we can conclude that autoantibody-positive and autoantibody-negative RA are also rather alike in the early arthritis phase when studied with MRI.

Long-term outcomes

Previous research into long-term outcomes in autoantibody-positive and autoantibody-negative RA revealed that autoantibody-positive patients have more damage progression, more swollen joints during follow-up and have a lower chance of achieving sustained DMARD free remission (SDFR).[19,24] Conversely, the pattern of joint involvement was similar and comparable PROs were described under treat-to-target treatment regimes.[19,25] However, the effect of treatment on long-term outcomes in autoantibody-positive and autoantibody-negative RA were scarcely studied.

To study effect of treatment on long term outcomes of autoantibody-positive and autoantibody-negative RA, logically, long term follow up is needed. Very long-term follow-up (>10y) is rare in randomized clinical trials (RCTs), as these are very costly. In the rare case that RCTs extend their follow-up to this time, treatment is often less strictly protocolized and more similar between arms, thereby making a RCT more comparable to a cohort study.[26] In this thesis, we took advantage of 25 years of follow-up of the Leiden EAC. To our knowledge, this is currently the largest observational cohort of RA.[27]

In **Chapter 7,** we found that disease activity improved in both patient groups. This was to be expected as the treat-to-target strategy, that is aimed at lowering DAS below a certain threshold, has been implemented around 2006. In contrast to the DAS, the other long-term outcomes (sustained DMARD-free remission, mortality, and functional disability) only improved in autoantibody-positive RA. This disconnection

between DAS and other long-term outcomes in autoantibody-negative patients is in stark contrast with the aim of treat-to-target strategies as the aim is to "lower the DAS on the short-term to enhance other outcomes on the long-term". Moreover, this disconnection implicates a different disease mechanism in autoantibody-negative RA. Also supporting the hypothesis of differences in disease mechanism, we observed that sustained DMARD-free remission and functional disability improved more in autoantibody-positive patients than in autoantibody-negative patients. While (changes in) treatment strategies were similar for autoantibody-positive and autoantibody-negative RA, improvement in long-term outcomes differed, again implying differences in different disease mechanisms.

In reaction to this study, one might argue that autoantibody-positive patients might have been treated more intensely before 2006, when treatment was less strictly aimed at a DAS target. If this would have been the case, one would expect less improvement in long-term outcomes with stricter treatment strategies after 2006 in autoantibody-positive RA. We observed the opposite, making it implausible that more intense treatment of autoantibody-positive patient before 2006 caused our results.

Therefore, we conclude that although disease activity has improved in both autoantibody-positive and autoantibody-negative RA, the response in long-term outcomes in recent decades with enhanced treatment strategies differed. Altogether, autoantibody-positive and autoantibody-negative RA seem rather different with respect to long-term outcomes and effect of treatment on long-term outcomes.

Mortality

In this thesis, we studied mortality in autoantibody-positive and autoantibody-negative RA patients in two different ways and found different results; In **Chapter 7,** we found that mortality significantly improved in autoantibody-positive RA whereas no significant improvement was found in autoantibody-negative RA. However, effect sizes were in the same direction and we observed no significant difference in mortality improvement between the two RA subtypes. Correction for age and gender was performed in these analyses but no adjustment for mortality in the general population was performed because excess mortality in RA is heavily dependent on follow-up duration and these follow-up durations differ between the cohorts studied.

In **Chapter 8,** we studied mortality corrected for the general population and follow-up duration. We found that mortality is normalized in ACPA-negative RA but not in ACPA-positive RA. Because standardized mortality rates cannot be compared between groups with a different age, gender and diagnosis-year distribution, comparisons between groups were not performed.[28]

Intuitively, these results might seem contradictory. However, the two chapters answer different questions: "Has mortality improved with enhanced treatment?" and "Is excess mortality still present with enhanced treatment?". An open question is whether excess mortality has improved since the introduction of enhanced treatment. However, to investigate this, two comparable large group of patients with similar age, gender and diagnosis-year distribution should be treated with either old or enhanced treatment strategies for >15 years. Unfortunately, this study is unfeasible and might also be unethical.

In conclusion, whether excess mortality has improved with enhanced treatment in autoantibody-negative RA is still to be debated. However, research into this subject might not be prioritized because excess mortality is less prominent in this group. In contrast, in autoantibody-positive RA, while mortality seems to have improved with enhanced treatment strategies, after longer follow-up excess mortality is still present. Therefore, research into treatment for excess mortality in autoantibody-positive RA is warranted. Still, with respect to the aim of this thesis, both studies show remarkable differences between autoantibody-positive and autoantibody-negative RA regarding to the long term outcome mortality.

IMPLICATIONS OF FINDINGS

Time to subdivide RA into type 1 and type 2

The aim of this thesis was to systemically study the differences between autoantibody-positive and autoantibody-negative RA from start of complaints to the end of disease. Previous research had already shown large differences between autoantibody-positive and autoantibody-negative RA before the start of complaints. We found that these disease types were rather similar in the phase from start of complaints to initial treatment response. In stark contrast, long-term outcomes and influence of treatment on long-term outcomes was very dissimilar. A graphical representation of this is presented in Figure 1. Altogether, we conclude that the differences between autoantibody-positive and autoantibody-negative RA before complaints and in long-term outcomes imply a (partly) different disease mechanism. Therefore, we propose that it is time to subdivide RA into autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2).

Implications of subdividing RA

If the hypothesis that type 1 and type 2 RA have (partly) different disease mechanisms is accepted, all previous research in RA should be revaluated and future research should be redirected. This is because risk factors and effect of treatment on outcomes might differ between the two types. And while correction for ACPA and/or RF has become

increasingly popular in research articles, stratification for autoantibody status in the only way to identify these differences.

In particular, as radiological damage and SDFR are more present in type 1 and type 2 respectively, studies with these outcomes might be primarily driven by one of the two disease types and cannot be generalised to the other type without further thought. Therefore, studies that used these outcomes and did not stratify for disease type should be revaluated. While doing this, it should be kept in mind that results of these studies might only apply to one disease type.

Finally, the 2010 classification criteria heavily load on the presence of autoantibodies. This is the result of the aim to early identify patients with persistent and/or erosive disease. Indeed, these criteria facilitated more early classification in type 1 patients.[29] However, the additional value of these criteria in type 2 patients is still to be elucidated and the need >10 affected joints (tender/swollen) to fulfill the 2010 criteria might have promoted classification of autoantibody-negative patients with more pain rather than patients with persistent and/or erosive disease. In the future, research could be aimed at identifying risk factors for persistent and/or erosive disease in autoantibody-negative early arthritis patients with a clinical diagnosis of UA. This with the ultimate aim to optimize early classification of type 2 RA.

Importance of type 2 RA

While RA research several decades ago predominantly focused on damage as an outcome, type 2 RA was originally seen as the mild subtype of RA and received less attention. As clinical relevant damage has become rare, PROs have become increasingly important.[30] Previous research has shown that with respect to PROs, type 2 RA is not a "mild" subtype. Also, in **Chapter 7,** we showed that with respect to long-term outcomes such as DAS, HAQ, mortality and SDFR, type 1 and type 2 are becoming increasingly similar. Therefore, type 2 RA has become less "mild" and research into type 2 is becoming increasingly important.

Another reason type 2 is becoming increasingly important is the rising prevalence of this RA subtype; In **Chapter 4**, we showed that the incidence of type 2 is rising, partly due to aging of the population. Also, we showed that disease duration has not shortened: In **Chapter 7,** we showed that SDFR rates did not rise in this type and in **Chapter 8**, we showed that excess mortality is no longer present in this RA type. Altogether, a rising incidence and a similar disease duration will result in a rising prevalence of type 2 RA. In contrast, type 1 RA will have a less prominent rising incidence due to aging. Type 1 also has improved mortality and improved SDFR and therefore will probably become less prevalent in comparison to type 2.

While type 2 is becoming less "mild" and more prevalent in comparison to type 1, less is known about this RA type and newer treatment strategies might be less effective in this RA type. With regard to treatment, in **Chapter 7** we showed that treatment has been intensified in this type but that this did not result in improvement of long-term outcomes. Therefore, when applying enhanced treatment strategies, doctors might be overtreating their type 2 patients.[31] Further research is needed to elucidate which treatment strategies do improve outcomes of type 2 patients.

With regard to pathophysiology, also less is known about type 2. While it is still debated whether autoantibodies play an active role in type 1 RA or are "innocent bystanders", [32] autoantibodies provide an anchor for pathophysiologic research in RA and therefore this research primarily focuses on type 1 RA, leaving a gap in knowledge about the pathophysiology of type 2 RA. Finally, with regard to diagnosis, the 2010 criteria are heavily dependent on autoantibodies and therefore the consequence of applying these criteria in type 2 patients has been insufficiently studied.

In conclusion, we want to emphasize that while type 1 RA is seen as the more severe type, type 2 RA is becoming increasingly prevalent and relatively more severe. Since less is known about type 2 in terms of optimal diagnosis, treatment strategies and pathophysiology, we want to advocate for more research into the optimal diagnosis, treatment and pathophysiology of type 2 RA.

Optimal division of type 1 and type 2

In this thesis, we promote the subdivision of RA into type 1 and type 2. However, how this division should exactly be performed should be based on future research. The division between autoantibody-positive and autoantibody-negative RA is most often based on RF, ACPA or both. Because these autoantibodies often cooccur, the resulting divisions are quite similar: in this thesis they were used interchangeably. RF+/ACPA- patients are generally older at onset compared to with RF+/ACPA+ patients, show similar incidence trends as RF-/ACPA- patients and have relatively milder damage progression.[33-37] In addition, RF is more prevalent in the general population.[38,39] Therefore, it might be more appropriate to make the subdivision between type 1 and type 2 strictly on ACPA.

Future research might result in even further subdivision of RA, especially of type 2 RA, since this type is suggested to be more heterogenous. Research into further subdivision might help to elucidate whether autoantibody level, number of autoantibodies or presence of other autoantibodies aid the optimal subdivision.[40-43] It is possible that other markers reflecting the underlying pathophysiology such as histology or metabolomic / lipidomic markers might help in making the best distinction. Ideally, the

division is made based on differences in pathophysiological mechanisms, but as long as these are unknown, epidemiological studies can be used. As the difference between type 1 and type 2 RA is most prominent pre-arthralgia and in long-term outcomes, these disease phases should be studied to elucidate what the optimal subdivision should be.

Tenosynovitis in type 1 and type 2 RA

Many studies described in this thesis show that tenosynovitis plays a prominent role in both type 1 and type 2 early RA: tenosynovitis predicts arthritis development in arthralgia patients, tenosynovitis is present in >80% of early RA patients and dissolving of tenosynovitis is associated with previous synovitis decrease. These results are interesting because tenosynovitis is a form of juxta-articular synovial inflammation and not intra-articular inflammation. Because RA is seen as a disease of the joints, intra-articular inflammation is historically associated with RA. However, also other forms of juxta-articular inflammation have been shown to play a role in early RA. Intermetatarsal bursitis is associated with early RA compared to other diagnoses.[44] The pathophysiology and the interaction of these juxta-articular and intra-articular forms of synovial inflammation remain to be elucidated in both RA types.

FINAL CONCLUSIONS

In short, based on this thesis, we learned that:

1. It is time to subdivide RA in autoantibody-positive RA (type 1) and autoantibody-negative RA (type 2) to enable stratified diagnosis, treatment and research in RA.
2. The prevalence of type 2 RA will rise due to increasing incidence, similar sustained DMARD-free remission rates and absence of excess mortality.
3. The goal to improve long-term outcomes by achieving remission on the short term has not been achieved in type 2 RA.
4. MRI-detected tenosynovitis is an early disease feature with high sensitivity and specificity for both type 1 and type 2 RA.

9

SUMMARY OF RESEARCH AGENDA

Type 1 and type 2 RA

- To systemically review RA studies that are stratified for autoantibody status to elucidate what is known about type 1 and type 2 RA, separately.
- To elucidate whether type 2 RA is indeed more heterogeneous and whether this type should be further subdivided.
- To develop a prediction model for persistence of autoantibody-negative early arthritis with the aim to reevaluate and maybe amend classification criteria in type 2 RA.
- To search for treatment strategies in type 2 RA that do not only decrease DAS but also improve long term outcomes.
- To optimize the distinction between type 1 and type 2 RA based on epidemiology pre-arthritis and in long-term outcomes, but also on other markers reflecting the underlying pathophysiology such as histology, metabolomics, lipidomics and autoantibody characteristics.
- To elucidate pathophysiological differences between type 1 and type 2 RA.

Tenosynovitis

- To examine the morphologic, histologic and molecular characteristics of tenosynovitis in early RA.
- To elucidate the etiology, interaction and timing of juxta-articular and intra-articular synovial inflammation in early RA.
- To further homogenize and validate scoring methods for tenosynovitis on MRI an ultrasound.
- To elucidate whether a tendon sheath is present around the extensor tendons at the MCP level and whether peritendinitis on MRI is in fact tenosynovitis.
- To further develop shorter and less costly MRI protocols to visualize tenosynovitis.

9

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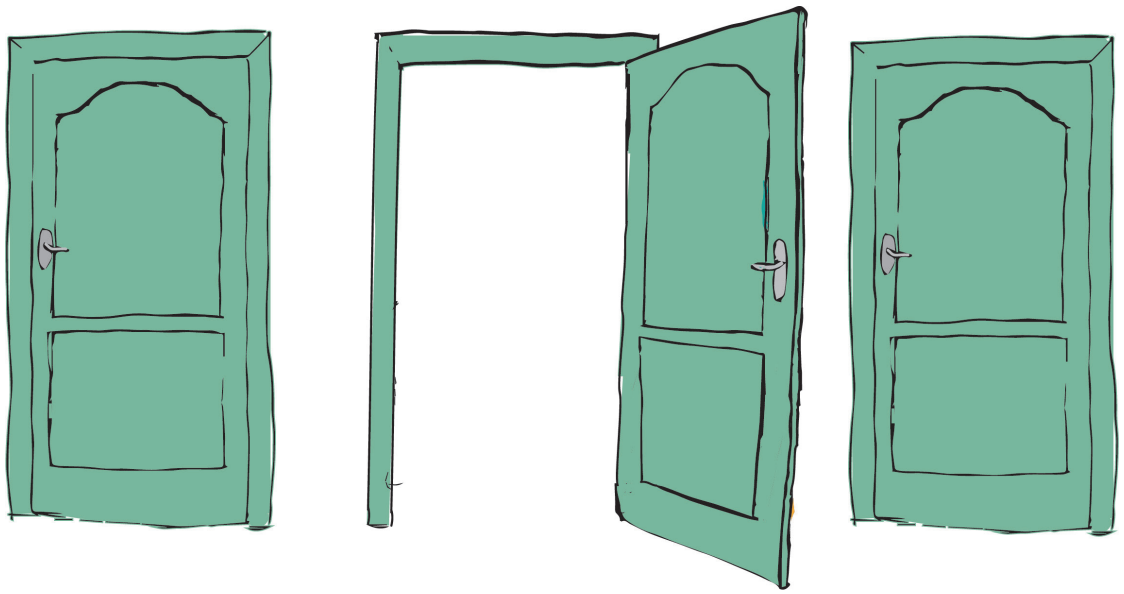
CHAPTER 10

Nederlandse samenvatting
Curriculum Vitae
List of publications
Dankwoord



NEDERLANDSE SAMENVATTING

In dit proefschrift hebben we gepoogd de verschillen en overeenkomsten tussen auto-antilichaam-positieve en auto-antilichaam-negatieve reumatoïde artritis te bestuderen. We bestudeerden deze ziekte vanaf het begin van de klachten tot het einde van de ziekte. We bestudeerden de symptomatische pre-artritisfase, de vroege artritisfase en langetermijnsuitkomsten van reumatoïde artritis-patiënten. We onderzochten deze fasen op gewrichtsniveau met MRI, op patiëntniveau met ziekteactiviteit en patiëntgerapporteerde uitkomsten en op samenlevingsniveau met behulp van gegevens van alle reumapatiënten uit de regio Leiden die zich sinds 1993 bij het LUMC hebben gepresenteerd.

Pre-artritis

In **hoofdstuk 2**, analyseerden we welke combinaties van MRI-kenmerken bij aanvang van klachten voorspellend waren voor reumatoïde artritis diagnose bij symptomatische patiënten zonder artritis. Dit deden we om ons begrip van de aangedane locaties in het begin van reumatoïde artritis te vergroten en om de voorspellende waarde van MRI te verbeteren. Dit onderzoek werd verricht in een uniek cohort van klinisch verdachte artralgie patiënten. We hebben vastgesteld dat MCP extensor peritendinitis een van de zeer vroege afwijkingen is bij reumatoïde artritis. Bovendien hebben we voorspellen van reumatoïde artritis diagnose verbeterd. Op basis van de voorspellers “aanwezigheid van MCP extensor peritendinitis” en “aantal locaties met subklinische ontsteking” hebben we vijf risicocategorieën gedefinieerd, waarvan de positief voorspellende waarde voor artritis ontwikkeling 67% in de hoogste categorie was. Daarna werden deze bevindingen gevalideerd in een onafhankelijke groep patiënten, met positief voorspellende waarde tot 63%. De volgende stap is om deze MRI eigenschappen te integreren met andere relevante biomarkers. Desalniettemin hebben we met dit onderzoek de risicostratificatie bij klinisch verdachte artralgie patiënten verbeterd en vergroot dit onderzoek ons begrip van de ontwikkeling van reumatoïde artritis.

Vroege artritis

In **Hoofdstuk 3** stelden we dat als MRI-detecteerbare tenosynovitis een echt reumatoïde artritis kenmerk is, de sensitiviteit voor reumatoïde artritis bij diagnose hoog zou moeten zijn, bij zowel bij auto-antilichaam-positieve als auto-antilichaam-negatieve reumatoïde artritis, en lager bij andere ziekten. We toonden aan dat de grote meerderheid (>80%) van de vroege reumatoïde artritis patiënten tenosynovitis heeft aan kleine hand- en voetgewrichten. Deze hoge sensitiviteit was aanwezig in zowel auto-antilichaam-positieve als auto-antilichaam-negatieve reumatoïde artritis, en de prevalentie was veel lager bij andere artritiden. Bovendien was de sensitiviteit van tenosynovitis voor reumatoïde artritis vergelijkbaar met de sensitiviteit van synovitis. Deze gegevens impliceren dat tenosynovitis, naast synovitis, een echt reumatoïde artritis kenmerk is. Dit begrip kan helpen bij toekomstig onderzoek naar de rol van

juxta-articulaire synoviale ontsteking in de pathogenese van zowel auto-antilichaam-positieve als auto-antilichaam-negatieve reumatoïde artritis.

In **Hoofdstuk 4** hebben we trends in de incidentie van auto-antilichaam-positieve en auto-antilichaam-negatieve reumatoïde artritis in de laatste 25 jaar in de Leidse regio bestudeerd. Onze hypothese was dat een deel van de toename in incidentie van auto-antilichaam-negatieve reumatoïde artritis wordt verklaard door de vergrijzing van de bevolking en dat dit in de toekomst zou kunnen leiden tot een toename van auto-antilichaam-negatieve reumatoïde artritis. Met behulp van gegevens van het Leidse Early Arthritis Cohort en bevolkingsgegevens uit de regio Leiden, vonden we een toenemende incidentie van auto-antilichaam-negatieve reumatoïde artritis, die afwezig was in auto-antilichaam-positieve reumatoïde artritis. Bovendien lieten we zien dat de toename van auto-antilichaam-negatieve reumatoïde artritis inderdaad gedeeltelijk wordt verklaard door de vergrijzing van de bevolking. Dit zal ervoor zorgen dat auto-antilichaam-negatieve reumatoïde artritis de komende jaren vaker voorkomt (geschatte toename van ~11% in 20 jaar) en benadrukt de behoefte aan onderzoek naar deze subgroep van reumatoïde artritis.

In **Hoofdstuk 5** bestudeerden we de relatie tussen MRI gedetecteerde ontsteking en vermoeidheid en vonden dat MRI ontsteking niet geassocieerd was met gelijktijdige vermoeidheid bij diagnose en tijdens ziekteverloop. Dit was zo bij zowel auto-antilichaam-positieve als auto-antilichaam-negatieve reumatoïde artritis patiënten. Bij het bestuderen van tijdsvolgorden zagen we dat een afname van MRI-ontsteking in het eerste jaar geassocieerd was met afname van vermoeidheid in het tweede jaar, maar de gestandaardiseerde effectgrootte was vergelijkbaar met klinische ziekteactiviteit zoals weergegeven met de disease activity score (DAS). Daarom helpt MRI-ontsteking niet bij het verklaren van vermoeidheid die niet door de DAS wordt verklaard. Dit suggereert dat er een plafondeffect is voor het verklaren van vermoeidheid door ontsteking en ondersteunt het idee dat vermoeidheid bij patiënten met reumatoïde artritis gedeeltelijk los staat van ontsteking. Daarnaast impliceren de resultaten dat het streven naar beeldvormingsremissie in plaats van klinische remissie de vermoeidheid niet vermindert bij auto-antilichaam-positieve en auto-antilichaam-negatieve reumatoïde artritis.

In **Hoofdstuk 6** hebben we patronen van afname van MRI-inflammatie bestudeerd bij 216 reumatoïde artritis en undifferatiated artritis patiënten die een vroege DMARD-behandeling kregen. We gebruikten cross-lagged modellen om de invloed van twee tijdspatronen te evalueren: een gelijktijdig patroon ("verandering in één ontstekingskenmerk is geassocieerd met gelijktijdige verandering in een ander kenmerk") en een volgend patroon ("verandering in één ontstekingskenmerk gaat vooraf aan verandering in een ander kenmerk"), in drie tijdsperioden (0-4 maanden, 4-12 maanden, 12-24 maanden). We zagen een gelijktijdige afname van synovitis, tenosynovitis en osteïtis. Bovendien ging de afname van synovitis vooraf aan de afname van tenosynovitis. Bij auto-antilichaam-positieve, maar niet bij auto-antilichaam-negatieve patiënten, ging een afname van synovitis ook vooraf aan afname van osteïtis. Derhalve waren patronen van verandering gedeeltelijk verschillend in de auto-antilichaam-positieve en auto-antilichaam-negatieve ziekte. Dit suggereert dat verschillende ontstekingsroutes ten grondslag liggen aan MRI-ontsteking bij auto-antilichaam-positieve en auto-antilichaam-negatieve reumatoïde artritis.

Resultaten op lange termijn

In **Hoofdstuk 7** hebben we het effect bestudeerd van behandelstrategieën, die de afgelopen 25 jaar zijn veranderd, op langetermijnsuitkomsten van auto-antilichaam-positieve en auto-antilichaam-negatieve reumatoïde artritis patiënten. We zagen dat reumatoïde artritis patiënten over de tijd vergelijkbaar waren gebleven, afgezien van eerdere diagnose. We ontdekten dat hoewel de ziekteactiviteit verbeterde bij zowel auto-antilichaam-positieve als auto-antilichaam-negatieve reumatoïde artritis-patiënten, de langetermijnsuitkomsten (de mogelijkheid om permanent te stoppen met medicatie, mortaliteit en functionele beperkingen) vooral verbeterden bij auto-antilichaam-positieve reumatoïde artritis-patiënten. De discrepantie tussen verbetering van ziekteactiviteit en daaropvolgende verbetering van langetermijnresultaten bij reumatoïde artritis zonder auto-antilichamen suggereert dat de onderliggende pathogenese van reumatoïde artritis met en zonder auto-antilichamen anders is. Op basis van onze gegevens denken we dat het tijd is om een differentiatie in reumatoïde artritis te maken en reumatoïde artritis te verdelen in auto-antilichaam-positieve (type 1) en auto-antilichaam-negatieve (type 2) types. Deze differentiatie zal zowel gerichte etiopathologische studies als gestratificeerde klinische studies stimuleren.

In **Hoofdstuk 8** wilden we de vraaag beantwoorden of de mortaliteit bij reumatoïde artritis genormaliseerd is, aangezien tegenstrijdige resultaten over dit onderwerp waren gepubliceerd. In veel van de onderzoeken naar mortaliteit tot nu toe, werd onvoldoende rekening gehouden met twee belangrijke factoren: follow-upduur en ziektesubtypes (zoals auto-antilichaam-positiviteit). Om de werkelijke impact van vroege intensieve behandeling op mortaliteit te bestuderen, hebben we een grote studie uitgevoerd (>1200 reumatoïde artritis patiënten) met een follow-up tot 25 jaar en voldoende power om te stratificeren voor follow-upduur en auto-antilichaamstatus. We toonden aan dat de oversterfte is verdwenen sinds de introductie van vroege intensieve behandeling bij auto-antilichaam-negatieve reumatoïde artritis, maar oversterfte een probleem blijft bij auto-antilichaam-positieve reumatoïde artritis.

Eindconclusies

Kortom, op basis van dit proefschrift hebben we geleerd dat:

1. Het is tijd om reumatoïde artritis onder te verdelen in auto-antilichaam-positieve reumatoïde artritis (type 1) en auto-antilichaam-negatieve reumatoïde artritis (type 2) om gestratificeerde diagnose, behandeling en onderzoek bij reumatoïde artritis mogelijk te maken.
2. De prevalentie van type 2 reumatoïde artritis zal stijgen als gevolg van een toenemende incidentie, vergelijkbare DMARD-vrije remissiecijfers en het ontbreken van oversterfte.
3. Het doel om de langetermijnsuitkomsten te verbeteren door op korte termijn remissie te bereiken, is niet bereikt bij type 2 reumatoïde artritis.
4. Tenosynovitis gevonden met MRI is een vroeg ziektekenmerk met een hoge sensitiviteit en specificiteit voor zowel type 1 als type 2 reumatoïde artritis.

CURRICULUM VITAE

Xanthe Marijn Edmée Matthijssen werd geboren op 29 juli 1992 in Rotterdam. In 2009 behaalde zij haar gymnasium diploma aan het Erasmiaans Gymnasium te Rotterdam.

Daarna behaalde zij eerst haar propedeuse wiskunde te Leiden, waarna zij in 2017 haar studie geneeskunde afrondde in dezelfde stad. In haar studietijd was zij erg actief op haar studentenvereniging SSR-Leiden, alwaar zij onder andere 2012-2013 een fulltime bestuursjaar als Quaestor Vereniging deed. Gedurende haar studie deed zij onderzoek op de afdeling epidemiologie van het Leids Universitair Medisch Centrum (LUMC) onder begeleiding van prof. dr. Dekker en op de afdeling reumatologie in hetzelfde ziekenhuis onder begeleiding van dr. Allaart.

Na haar studies, startte zij met haar promotieonderzoek op de afdeling reumatologie van het LUMC onder begeleiding van prof. dr. A.H.M. van der Helm – van Mil. Zij combineerde dit promotie onderzoek met het behalen van de researchmaster Statistical Science for the Life Sciences.

In mei 2021 is zij gestart met haar opleiding tot reumatoloog in regio Leiden. Momenteel volgt zijn de vooropleiding interne geneeskunde in het HMC in Den Haag (opleider dr. A. Bootsma). Zij is getrouwd met Juliette Kamp en woont in Leiden.

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