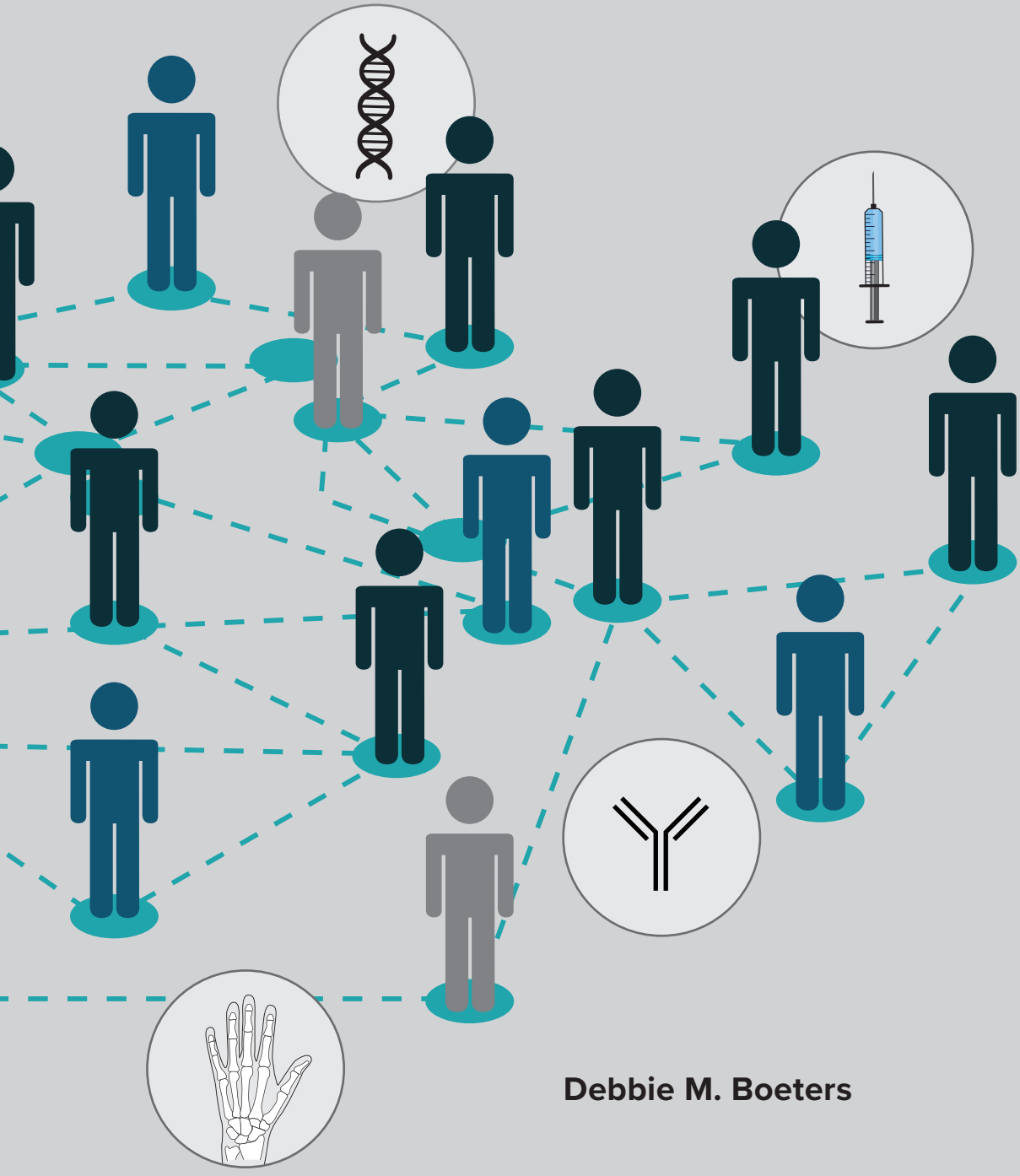
EARLY IDENTIFICATION AND RESOLUTION OF RHEUMATOID ARTHRITIS



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Early identification and resolution of rheumatoid arthritis

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The studies described in this thesis were performed at the Department of Rheumatology at the Leiden University Medical Centre, Leiden, the Netherlands.

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Early identification and resolution of rheumatoid arthritis

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Contents

Chapter 1	General introduction	9
PART I	**Early recognition of rheumatoid arthritis**	**25**
Chapter 2	Which patients presenting with arthralgia eventually develop rheumatoid arthritis? The current state of the art	27
Chapter 3	The 2010 ACR/EULAR criteria are not sufficiently accurate in the early identification of autoantibody-negative rheumatoid arthritis: Results from the Leiden-EAC and ESPOIR cohorts	57
Chapter 4	Does information on novel identified autoantibodies contribute to predicting the progression from undifferentiated arthritis to rheumatoid arthritis? A study on anti-CarP antibodies as example	71
Chapter 5	Are MRI-detected erosions specific for RA? A large explorative cross-sectional study	81
Chapter 6	Evaluation of the predictive accuracy of MRI-detected erosions in hand and foot joints in patients with undifferentiated arthritis	103
PART II	**Clinical and imaging features and the ACPA response**	**111**
Chapter 7	MRI-detected osteitis is not associated with the presence or level of ACPA alone, but with the combined presence of ACPA and RF	113
Chapter 8	The prevalence of ACPA is lower in rheumatoid arthritis patients with an older age of onset but the composition of the ACPA response appears identical	135
PART III	**Resolution of rheumatoid arthritis**	**159**
Chapter 9	Does immunological remission, defined as disappearance of autoantibodies, occur with current treatment strategies? A long-term follow-up study in rheumatoid arthritis patients who achieved sustained DMARD-free status	161

Chapter 10	Subdividing ACPA-negative RA: patients with high likelihood of achieving sustained DMARD-free remission are characterized by a combination of serological markers at disease presentation	185
Chapter 11	Summary and discussion	207
Chapter 12	Nederlandse samenvatting	227
Appendices	Curriculum Vitae	238
	List of publications	239
	Dankwoord	242

1

General introduction

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic disease, characterized by inflammation of the joints that may lead to structural damage. Patients with RA typically present with pain, swelling and morning stiffness of the small joints of the hands and feet. Besides the joints, many other organs can be involved which might be notable by systemic symptoms such as fatigue and weight loss.[1-3] Subsequently, RA results in significant morbidity with functional and work disability and systemic complications, resulting in high socio-economic costs.[4-6] With a prevalence of 0.5-1% in developed countries, RA is among the most common rheumatic diseases. It is three times more prevalent in women than in men and its incidence increases with age.[6]

Although the pathogenesis of RA is not fully elucidated, it is known that there is a complex interplay between genetic susceptibility, environmental factors and an abnormal (auto) immune response with involvement of both the innate and the adaptive immune system. The results is thickening of the synovial layer of joints with infiltration of fibroblast-like and macrophage-like synoviocytes, macrophages, T cells and B cells. Synovitis is perpetuated by positive feedback loops eventually leading to cartilage degradation and bone erosions and eventually to systemic disorders.[6,7]

The two most important autoantibodies involved in RA are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). RF is a polyclonal antibody directed against the Fc portion of immunoglobulin G and is the first autoantibody that was described in RA. RF is present in about 60% of early RA patients, nonetheless RF is also frequently observed in other rheumatic diseases and in patients with chronic infections.[8-11] ACPA are directed against proteins or peptides containing citrulline, which is an uncharged amino acid generated by a post-translational modification of the positively charged amino acid arginine by peptidyl arginine deiminases.[12] ACPA are present in 50-60% of early RA patients and in 60-90% of patients with long-standing disease and are observed in only 1% of the general population which makes them highly specific for RA.[13-15] Both ACPA and RF can be present years before disease onset and together with the observed strong association between ACPA and RA, this suggests that ACPA have a pathogenic role in RA although the underlying mechanism remains unclear.[16-18] Both ACPA and RF are associated with relevant outcomes such as radiographic progression and are used as diagnostic tools and included in RA classification criteria.[19,20] Nonetheless,

in approximately one third of RA patients both these autoantibodies are lacking.[21] Therefore, research is focused on exploring other, novel autoantibodies to also identify these patients.

Besides autoantibodies, genetic risk factors are important for the development of RA. More than 50% of the risk of RA is attributable to genetic risk factors, both in ACPA-positive and ACPA-negative RA.[22] The HLA class II molecules account for about 11-37% of the total genetic effect, and are therefore considered the major genetic risk factor of development of RA, especially in ACPA-positive patients.[23,24] Several other genetic risk factors have been identified which are different between ACPA-positive and ACPA-negative RA.[25,26] Finally, several environmental factors have been described which affect susceptibility for RA, such as smoking, diet and socioeconomic status.[27] Of these factors, smoking appears to be the most important environmental risk factor, especially in ACPA-positive patients carrying HLA SE alleles.[28,29]

Classification of RA

The diagnosis of RA is made by the rheumatologist, based on clinical, laboratory and imaging observations. Diagnosing RA is basically pattern recognition. Since there is neither an exact or simple definition of RA, nor a diagnostic test, this presumably results in a heterogeneous group of RA patients. To compare findings of studies of RA patients, it is important to identify a relatively homogenous group of RA patients; therefore classification criteria have been developed.

In current studies both the 1987 ACR criteria and the 2010 ACR/EULAR classification criteria for RA are used; a comparison of both criteria is shown in Table 1.1.[20,30] The 1987 ACR criteria were derived to increase specificity compared to the previously used 1958 criteria, thus to improve differentiation of patients with established RA from patients with other rheumatic diseases.[30] Although the 1987 criteria indeed have shown to be specific in classifying established RA, identification of patients with early RA is lacking.[31] Nowadays, it is clear that early identification and treatment of RA patients is important to improve clinical outcomes and prevent joint damage progression.[32-34] There might even be an early phase in the disease in which the disease is more susceptible to treatment, presumably because of not fully matured underlying disease processes.[35] With the knowledge that early treatment is beneficiary in RA, clinical trials with early RA patients were performed.

To facilitate the identification of early RA patients which was needed for these studies, the 2010 ACR/EULAR criteria have been developed.[20] The major difference between the 2010 criteria and the previous 1987 criteria, is that the 2010 criteria focused on features in early arthritis that associated with persistent and/or erosive disease, and therefore acute phase reactants and ACPA were added. Rheumatoid nodules and the presence of radiographic erosions, which were included in the 1987 criteria, were not included anymore in the 2010 criteria because these are both characteristics of established disease. However, to prevent that patients with inactive disease are misclassified as having no RA, the presence of erosions typical of RA can be used as prima facie evidence of RA, precluding the need of fulfilling the criteria.[36]

Table 1.1 Comparison of classification criteria for RA

1987 ACR criteria	2010 ACR/EULAR criteria	
	Target population: patients with at least 1 joint with clinical synovitis, not better explained by another disease	points
1. Morning stiffness ≥1 hour	Joint involvement*	
2. Arthritis of ≥3 joint areas#	1 large joint	0
3. Arthritis of hand joints	2-10 large joints	1
4. Symmetric arthritis	1-3 small joints	2
5. Rheumatoid nodules	4-10 small joints	3
6. Serum RF	>10 joints (at least 1 small joint)	5
7. Radiographic changes	Serology	
	Negative RF and negative ACPA	0
	Low-positive RF or low-positive ACPA	2
	High-positive RF or high-positive ACPA	3
	Acute-phase reactants	
	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
	Duration of symptoms	
	<6 weeks	0
	≥6 weeks	1
≥4 out of 7 criteria must be present for classification of RA. Criteria 1-4 must have been present for ≥6 weeks. #Left or right PIP, MCP, wrist, elbow, knee, ankle and MTP	≥6 out of 10 points needed for classification of RA. *refers to any swollen or tender joint on examination Large joints: shoulders, elbows, hips, knees, ankles. Small joints: MCP, PIP, 2nd-5th MTP, thumb IP, wrists.	

Several studies have indeed shown that the 2010 criteria are fulfilled earlier in time than the 1987 criteria at the expense of a slight decrease in specificity.[37] When using these classification criteria, it is important to realize that the phenotype of RA patients at disease presentation and during the disease course is different between both criteria sets.[38]

Patients with arthritis who do not fulfil RA classification criteria and who do not have another diagnosis explaining their symptoms are referred to as patients with undifferentiated arthritis (UA), and therefore the diagnosis 'UA' is made per exclusionem.

Treatment of RA

In the last decades, the treatment of RA has improved tremendously. Whereas treatment consisted initially of non-steroidal anti-inflammatory drugs and delayed treatment with disease-modifying antirheumatic drugs (DMARDs), nowadays the treatment armamentarium has increased and in addition patients are treated earlier, directly after diagnosis with RA. Another improvement in the treatment of RA was the incorporation of disease activity scores (DAS) to monitor the disease, which has contributed to improved patient outcomes.[39]

According to current guidelines, treatment should be aimed at reaching sustained remission or low disease activity.[40] Methotrexate in combination with glucocorticoids as bridging therapy is recommended as initial treatment strategy. When this treatment failed, another conventional DMARD should be considered. Use of a biological DMARD is recommended when patients failed on two or more conventional DMARDs. The ultimate treatment goal is achievement of sustained remission which is increasingly observed over the past years.[41] Some of the patients in sustained remission can even successfully taper and stop all DMARD therapy; these patients are considered to be in a sustained DMARD-free status. This outcome is also relevant from a patient perspective, since this status is characterized by normalization of functional status and lower levels of fatigue, pain and morning stiffness.[42] It is currently considered the best possible outcome of RA. Only a few factors are known to be associated with a sustained DMARD-free status, which are short symptom duration at disease presentation and the absence of autoantibodies.[9,43] The biological processes underlying extinguishment of disease in patients who achieve sustained DMARD-free remission are unclear.

Stages of RA development

Since it has become clear that early treatment of RA is needed to improve patient outcomes, research has focused on the earliest stages of disease, even before arthritis has developed. It is ascertained that RA has a preclinical disease period since autoantibodies, such as ACPA and RF, can be present years before the onset of disease and there is data indicating that the ACPA immune response matures during this preclinical phase.[17,18,44-48] Furthermore, markers of systemic inflammation can be increased in the preclinical disease phase.[49,50] To facilitate comparison between studies in these early disease phases, a EULAR study group described different phases before the development of RA which are genetic risk factors, environmental risk factors, systemic autoimmunity associated with RA, symptoms without clinical arthritis, unclassified arthritis and RA (Figure 1.1).[51] These phases can be used in a combinatorial manner, thus patients can be in two phases at the same time. Furthermore, the different phases do not occur in all patients who will develop RA and also do not occur necessarily in the same order. Importantly, patients with pre-RA can only be identified retrospectively, once it is known that patients have progressed to RA as the majority of patients with certain risk factors will never develop RA.

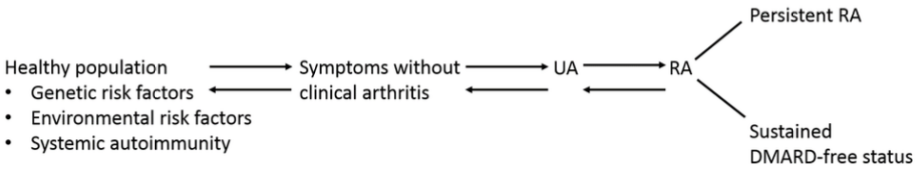

Figure 1.1 Different phases of RA development

The phase in which clinical synovitis can be identified is that of UA. Of the patients with UA, about half will achieve spontaneous remission, whereas RA develops in one-third.[52] This disease state is of special interest because when risk factors for progression to RA are known, patients with RA can be identified even earlier. This has been studied before and a prediction model with high discriminative ability has been developed and validated which includes several clinical features, autoantibodies and C-reactive protein.[53,54] However, this prediction model was developed in patients with UA according to the 1987 criteria, while characteristics at disease onset of UA patients according to the 2010 criteria are different.[55] In

addition, when using the 2010 criteria, accurate predictors of progression for ACPA-negative UA patients are lacking.

Besides early identification of UA patients, the aim is currently to identify patients with imminent RA even earlier, in the phase of Clinically Suspect Arthralgia (CSA). Patients with CSA have recent-onset arthralgia that is considered at risk of RA based on the clinical expertise of the rheumatologist.[56] Identification of CSA patients who will develop RA is challenging as the majority of patients will never develop RA. Several factors have been associated with development of arthritis in patients with CSA, such as the presence of autoantibodies and subclinical joint inflammation detected by magnetic resonance imaging (MRI), but this has not resulted yet in a validated prediction model which can be used in clinical practice.[57,58] Perhaps other biomarkers than autoantibodies and imaging markers are needed for accurate risk stratification.

In addition to predicting which patients with CSA or UA will progress to RA, it is important to prevent this progression since the majority of RA patients have persistent disease and require life-long treatment. Whether therapeutic intervention in the phase of CSA or UA is helpful in preventing RA development is still incompletely clarified and is subject of current research.[59]

Heterogeneity of RA

Most likely, RA can be considered as a syndrome consisting of different disease entities which are all characterized by chronic synovial inflammation, but differ in underlying pathophysiology and disease outcome. Currently, the most common used subdivision is that into ACPA-positive and ACPA-negative RA. These two subsets have different disease courses and the etiology is different regarding both genetic and environmental risk factors. But even ACPA-positive and ACPA-negative RA might be differentiated further into subgroups. The pathogenesis and risk factors of ACPA-negative RA are much less understood than of ACPA-positive RA, but presumably ACPA-negative RA is more heterogeneous than ACPA-positive RA. The assumption that ACPA-negative RA consists of subgroups is supported by the observation that part of the ACPA-negative RA patients have a destructive disease, while others have not.[60] Previously, it was studied whether ACPA-negative RA patients could be subdivided into clinically distinguishable sub phenotypes but no clear sub phenotypes were identified.[61] Perhaps, it is possible that such

sub phenotypes are observed when other factors than clinical ones are assessed. Deciphering the syndrome of RA into subgroups is not only important to get more pathogenic insight into the disease but it might also eventually improve patient outcomes by personalizing treatment.

Aim and outline of this thesis

In general this thesis has three main aims:

1. To improve identification of early RA patients
2. To investigate clinical and imaging features in relation to the autoantibody response
3. To improve the understanding of mechanisms underlying a sustained DMARD-free status

This thesis contains three parts.

In **Part I**, the association between several biomarkers and the development of RA was studied. Early treatment initiation of RA patients is important because it is associated with improved outcomes. Therefore, it is important to perform research in the preclinical phase of RA. In **Chapter 2**, it is reviewed what is currently known about the phase of pre-clinical RA. The relevance of serological and imaging markers for predicting progression to arthritis and their potential value in prognostication is discussed. Both autoantibody-positive and autoantibody-negative RA patients should be identified early in the disease process, because early treatment is beneficiary in both disease subsets.[34,35] In **Chapter 3**, it was studied whether the 2010 ACR/EULAR criteria performed equally well in early classification of autoantibody-positive and autoantibody-negative RA. Although the 2010 criteria were developed for early classification of RA patients, in daily practice it may sometimes be used in the diagnostic process. In the 2010 criteria the presence of ACPA and RF are included, but one third of RA patients are negative for both of these autoantibodies, and therefore require more than 10 involved joints to fulfil classification criteria. The past years several novel autoantibodies have been identified, such as anti-carbamylated protein antibodies and anti-acetylated peptide antibodies.[62,63] The additional value of these autoantibodies for the early identification of RA remains unclear. In **Chapter 4**, it is discussed whether information on novel autoantibodies might contribute to the earlier identification of RA. As an example, the additional value of anti-CarP antibodies was studied. Besides autoantibodies, the additional

value of imaging biomarkers for improving early identification of RA patients is subject of several studies. Traditionally, radiographs are frequently used in the diagnostic process for detecting structural damage, including erosions and joint space narrowing. Recently it was recommended to use MRI for this purpose because MRI is more sensitive in detecting erosions than radiographs.[64] In **Chapter 5** we studied several characteristics of MRI-detected erosions in RA patients. The specificity of MRI-detected erosions for RA was determined by comparing RA patients with patients with other types of arthritis and with healthy controls. A subsequent and clinically relevant question is whether MRI-detected erosions in patients presenting with UA are valuable in predicting the progression to RA; this was studied in **Chapter 6**.

In **Part II**, the association between clinical and imaging features and the autoantibody response was investigated. An advantage of MRI over conventional radiographs is that MRI can visualize inflammatory soft tissue changes, such as synovitis and tenosynovitis. In addition, bone marrow edema (BME) can be depicted which are lesions due to replacement of bone marrow fat by an inflammatory cell infiltrate, reflecting osteitis. The presence of BME is associated with erosive progression.[65] Since autoantibodies are also a strong predictor of erosive progression of which the underlying mechanism is unclear, we investigated the association between autoantibodies and MRI-detected BME in **Chapter 7**. Next, the association between clinical features and the ACPA response was studied. Several studies have shown that RA patients with a disease onset at older age have different disease characteristics than patients presenting at younger age, possibly indicating the presence of different disease subsets. In **Chapter 8**, the association between the presence of three different autoantibodies (ACPA, RF and anti-CarP antibodies) and several clinical parameters and age of onset of RA was studied in five different early arthritis cohorts with the ultimate aim to identify subgroups of RA patients. Besides mere presence of ACPA, also several characteristics of the ACPA immune response were studied in relation to age.

In **Part III**, the focus was on the long-term outcome of RA patients. To this end, the achievement of a sustained DMARD-free status was studied. Only a minority of patients is able to achieve this outcome and biological processes underlying extinguishment of disease are unclear. Previously, it was suggested that autoantibodies act as a driving force for persistent inflammation in RA and therefore, that disappearance of autoantibodies is associated with the highest likelihood of achieving sustained DMARD-free remission.[66] However,

the association between disappearance of ACPA and RF and sustained remission has not been extensively studied before and was studied in **Chapter 9**. To this end, anti-CCP2 IgG and IgM and RF IgM levels were measured at diagnosis of RA and around the time of achievement of sustained DMARD-free remission. If autoantibodies are underlying achievement of remission, these might disappear when patients are clinically cured. The fact that only a minority of patients is able to achieve a sustained DMARD-free status, supports the presumption that RA is a heterogenous disease. Besides clinical characteristics and autoantibodies, other serological biomarkers might help in differentiating RA in subgroups. In **Chapter 10**, twelve proteins were measured at disease onset, and it was assessed whether the presence of these proteins contributes to the identification of subgroups of RA patients, for which sustained DMARD-free remission is an achievable outcome.

The results of the studies performed in this study are summarized and discussed in **Chapter 11**.

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PART I

Early recognition of rheumatoid arthritis

2

Which patients presenting with arthralgia eventually develop rheumatoid arthritis? The current state of the art

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Abstract

Early initiation of treatment in patients with inflammatory arthritis at risk of persistence and/or erosive progression is important because it is associated with a reduced rate of progression of joint damage and functional disability. It has been proposed that a window of opportunity exists, during which disease processes are less matured and disease modification can be more effective. The phase of arthralgia preceding clinical arthritis is likely to be an important part of this window of opportunity, during which treatment might prevent progression to clinical arthritis. Several proof-of-concept trials in individuals with arthralgia are now evaluating this hypothesis. Central to such trials is the ability to identify groups at high risk of rheumatoid arthritis (RA) in whom preventive treatment can be tested. This review describes the relevance of adequate prediction making, as well as the accuracy of different types of predictors (including imaging and serological markers) with their value in predicting the progression of arthralgia to arthritis. Despite promising results, studies have been performed in heterogeneous patient populations and most findings have not been validated in independent studies. Future observational or preventive studies should be conducted with homogeneous patient groups (e.g., patients fulfilling the European League Against Rheumatism criteria for arthralgia at risk of RA) in order to increase interstudy comparability and to allow result validation.

The relevance of adequate prediction making

Research into the earliest phases of rheumatoid arthritis (RA) is important because early treatment is associated with better outcomes. To facilitate this research the European League Against Rheumatism (EULAR) study group of risk factors for RA has defined several stages of RA development: genetic risk factors for RA, environmental risk factors for RA, systemic autoimmunity associated with RA, symptoms without clinical arthritis and unclassified arthritis (UA).[1] These stages are based on the presumed order in which different risk factors exert their effects. Individuals in the first three stages are generally asymptomatic. Over time symptoms may develop - initially often in the absence of clinically evident arthritis. In patients with established RA, the different phases may be identified retrospectively. However, it is clinically important to be able to predict with accuracy and confidence the future development of RA during its prearthritis stages. During recent years the phase of arthralgia has gained increasing interest as the risk of progression to RA is (in most cases) likely to be higher in symptomatic than in asymptomatic 'at risk' individuals. In addition, this is the way individuals typically present to medical care.

The phase of arthralgia is likely to be an important part of the so-called window of opportunity. Studies in patients with classified RA have revealed that an earlier start of treatment is associated with better outcomes.[2,3] Because at presentation with clinical arthritis most patients will have a chronic disease, it is hypothesised that the period preceding clinical arthritis might be important. Within this prearthritis phase, disease processes might be less matured, making patients more susceptible to disease-modifying antirheumatic drugs (DMARDs). A review of murine studies suggested that DMARD initiation (e.g., methotrexate and abatacept) prior to clinical arthritis was effective.[4] Several ongoing proof-of-concept trials in individuals with arthralgia are evaluating the hypothesis that DMARD initiation can prevent progression to clinically evident arthritis. Results of two randomised controlled trials have been published; the first included 83 patients with anti-citrullinated protein antibodies (ACPA)-positive and/or rheumatoid factor (RF)-positive arthralgia who were treated with dexamethasone or placebo, and the second included 82 patients with ACPA-positive and RF-positive arthralgia with C-reactive protein (CRP) levels ≥3 mg/L and/or subclinical synovitis on ultrasound (US) or magnetic resonance imaging (MRI) of the hands, who were treated with a single infusion of rituximab or placebo.[5,6] Although a decrease in ACPA levels and a delay in arthritis onset were reported, neither intervention prevented the

development of RA. This failure to prevent RA development may indicate that (1) the hypothesis is false (i.e., that the disease is not more modifiable in its arthralgia phase compared with its arthritis phase), or (2) the wrong drugs were tested, or (3) the studies included too few patients with a high risk of progression to RA, making it less easy to observe a preventive effect.

The importance of including patients with a high risk of progression to RA was illustrated in a recent post-hoc analysis of data from the Probable Rheumatoid Arthritis: Methotrexate versus Placebo Treatment (PROMPT) trial, in which patients with UA were treated with methotrexate with the aim of preventing progression to RA.[7] The risk of progression to RA was ~30%, and without further stratification, methotrexate did not modify this risk. However when only patients with a high (>80%) 1-year predicted risk of progression to RA were evaluated, methotrexate was highly effective in preventing RA development. In addition, methotrexate was also associated with DMARD-free remission in this high-risk group (36% vs. 0% in the placebo group). Although these post-hoc analyses were based on small sample sizes, these data demonstrate the relevance of including patients with a sufficiently high risk in preventive trials. The results of ongoing proof-of-concept trials in arthralgia are awaited over the next decade.

Not all of the ongoing preventive trials have fulfilment of the 2010 classification criteria for RA as primary outcome. This is supported by the fact that the presence of persistent clinical arthritis or a clinical diagnosis of RA is an outcome that fits with daily clinical practice.

Before implementing potential positive findings of preventive trials in daily rheumatological practice, we need to know which patients with arthralgia would otherwise develop RA and should be offered treatment, and conversely which patients should be reassured that disease progression is unlikely (Figure 2.1).

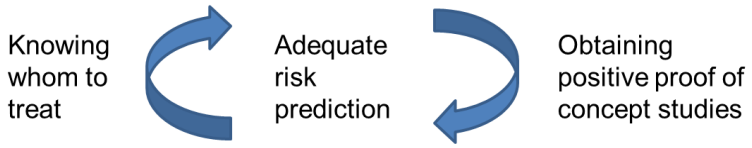

Figure 2.1 Adequate risk prediction is crucial for the design of informative preventive trials and for implementation of positive trial results

Types of predictors

Optimally performing biomarkers are often causally related to the underlying biological process. Examples include the combination of increased free thyroxine (FT4) and decreased serum thyrotropin (TSH) levels, which are pathognomonic for hyperthyroidism, and the urinary human chorionic gonadotropin (HCG)-based pregnancy test, which is seldom negative in pregnant women and high HCG levels are rarely present in settings other than pregnancy. Predictors can also be bystanders, markers that are side products of the biological process but characteristic of the disease. Other predictors are phenotypic in nature (Figure 2.2). RA has a complex aetiopathology and its development is not easily reflected by a single marker. The presence of ACPA within RA is strongly predictive of erosive progression and may be causally related to the development of bone erosions, but its role in the development of RA is unclear and its presence is not 1:1 related to disease development. Furthermore, it has become clear that in addition to RF and ACPA, several other autoantibodies are present in RA.[8-10] These different sets of autoantibodies do not seem to relate to specific (sub)phenotypes of RA and may thus be considered as bystanders, although very useful in the diagnostic process.[11] In the absence of pathognomonic markers, multiple biomarkers should be combined to predict which patients with arthralgia will progress to RA.

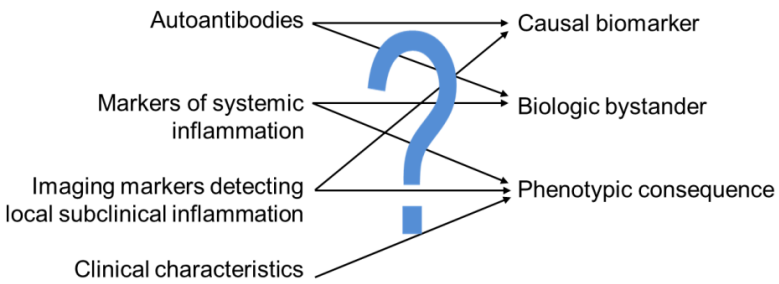

Figure 2.2 Predictors of rheumatoid arthritis development belong to different categories

A predictor of disease might directly reflect the underlying biological process, it can be a biological bystander of disease, or it might have no relation at all with the underlying biology and is a phenotypic marker.

Differentiating arthralgia suspicious for progression to RA from other arthralgias

Before reviewing the accuracy of different types of predictors, appreciation of the population studied is important. Arthralgia is a non-specific symptom and the biological nature of joint pain is diverse. Consequently, the risk to progress to RA is different for patients with arthralgia in different settings.

Musculoskeletal (MSK) symptoms are very prevalent in primary care.[12] Primary care data from the Netherlands suggest an annual incidence of non-traumatic MSK symptoms of ~300/1000.[13-15] In other words, almost one-third of the population visits the general practitioner (GP) at least once a year with an MSK symptom. The vast majority of these patients have explanations for their joint symptoms other than the beginning of a systemic inflammatory arthritis, and inflammatory arthritis is considered by GPs in only a minority of patients (Figure 2.3). A separate Dutch GP study recorded an incidence of suspected arthritis of ~3/1000/year; most patients had a monoarthritis, and 60% had self-limiting symptoms.[16] A small proportion of patients had suspected oligoarthritis or polyarthritis, and symptom persistence was more common in this group. These data support the notion that GPs are able to differentiate inflammatory from non-inflammatory cases of MSK symptoms and that the incidence of suspected inflammatory arthritis in primary care is low.

A similar observation has been made in secondary care. Most patients with arthralgia referred to rheumatologists have a diagnosis other than (imminent) RA. In addition, of patients presenting with arthralgia of uncertain cause, the large majority are not considered to be at risk of RA by their rheumatologists. A recent study revealed that only 7% of these patients with arthralgia were identified as clinically suspicious for progression to RA (clinically suspect arthralgia, CSA).[17] Importantly, for patients with CSA, the odds for progression to RA were 55 times larger than the odds for patients with unexplained arthralgia. The rheumatologists' clinical expertise had a high accuracy (93%), sensitivity (80%) and specificity (93%) for future RA. Although these data support the use of the rheumatologist's clinical experience in identifying patients with arthralgia who are at risk of RA, a drawback is that this approach is subjective. This is a particular problem for research studies, where homogeneous groups of patients should be included. A EULAR task force has recently explicated this clinical expertise in clinical items that are measurable.[18] The resulting EULAR definition of arthralgia suspicious for progression to RA consists of seven clinical items and can be used in patients with

arthralgia in whom imminent RA is considered the most likely explanation for the symptoms (Figure 2.4). The definition was validated in the rheumatological practices of 18 European rheumatologists (area under the curve: 0.92) with clinical expertise as the reference. The first longitudinal study of patients with CSA showed that the definition had a high sensitivity and served to further harmonise patients, as patients with arthralgia who were identified as CSA by their rheumatologist but had <3 clinical items indeed had a lower risk of progression to RA.[19]

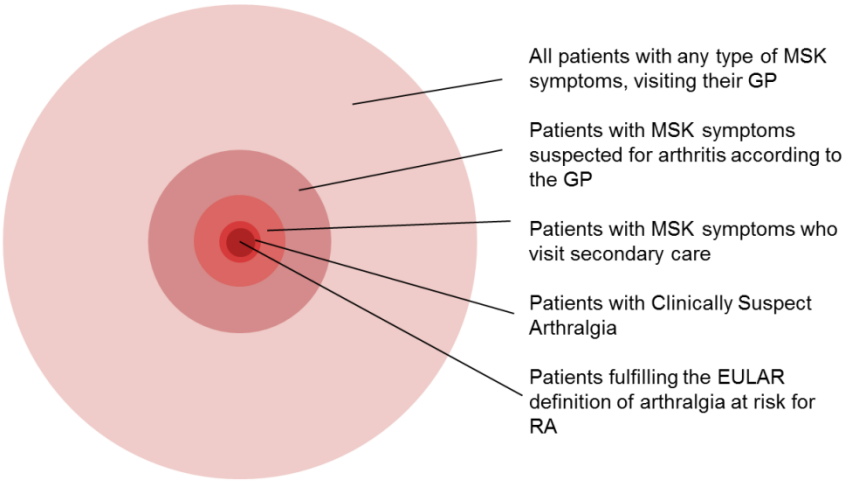

Figure 2.3 Clinical expertise of GPs and rheumatologists in differentiating patients with arthralgia

This figure is constructed based on the following references: The clinical expertise of GPs and rheumatologists is effective in differentiating patients with arthralgia; of all patients with MSK symptoms visiting their GPs (~300/1000/year),[13–15] only a small subset is suspected for arthritis (~3/1000/year).[16] Of all patients with any MSK symptoms visiting secondary care (~8/1000/year),[53] only 7% were identified as CSA.[17] The incidence of any MSK symptom in secondary care is higher than the incidence of patients with suspected arthritis in primary care, as GPs also refer patients with MSK symptoms in whom they did not suspect arthritis to be present. 74% of patients with CSA had a positive EULAR definition.[19] CSA, clinically suspect arthralgia; EULAR, European League Against Rheumatism; GP, general practitioner; MSK, musculoskeletal symptoms; RA, rheumatoid arthritis.

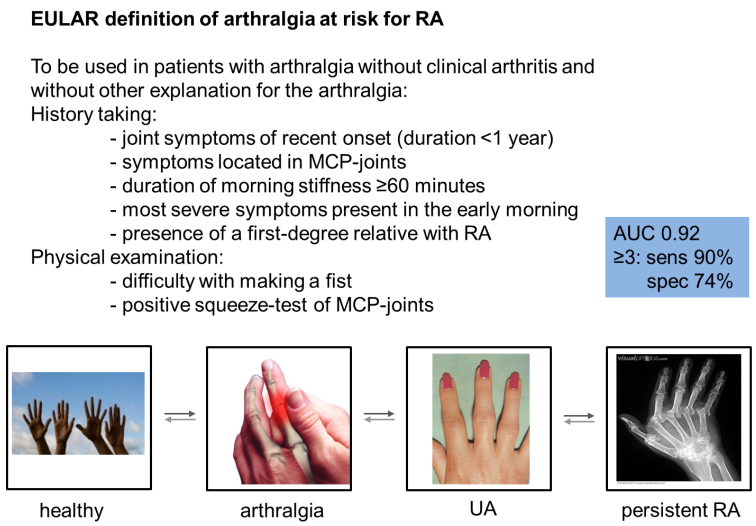

Figure 2.4 EULAR-defined characteristics describing arthralgia at risk for RA

The reported AUC, sensitivity and specificity were calculated within newly presenting patients with CSA in outpatient clinics of European expert rheumatologists (who were part of the task force who defined arthralgia at risk for RA) with clinical expertise as reference.[18] A sensitive definition requires the presence of at least three items and a specific definition requires the presence of at least four items. AUC, area under the curve; EULAR, European League Against Rheumatism; MCP, metacarpophalangeal; RA, rheumatoid arthritis; sens, sensitivity; spec, specificity; UA, undifferentiated arthritis.

Altogether, patients with arthralgia in secondary care who are considered as CSA and fulfil the EULAR definition of arthralgia represent a very small proportion of all individuals suffering from joint pain (Figure 2.3). An optimised selection of patients with arthralgia will result in an increased risk of RA in the population, and - as a result of Bayes' theorem - this will also result in higher post-test chances when performing additional tests, such as laboratory or imaging tests, in this subset of patients with arthralgia.

Search strategy

The accuracy of different types of laboratory or imaging markers for predicting RA development is reviewed below. With the assistance of a medical librarian, we searched in the medical literature databases PubMed, Embase (Ovid version), Web of Science and Cochrane Library up to June 2017. Central terms in our search strategy were arthralgia, arthritis, autoantibodies, serological markers and imaging. In total 145 references on autoantibodies, 117 on serological markers and

310 on imaging markers were extracted. Reference lists of the identified articles were hand-searched for additional articles. From the total list of references, we selected the studies on patients with arthralgia with a longitudinal cohort design.

The predictive accuracy of autoantibody testing in arthralgia

Nested case-control studies have shown that autoantibodies can be present years before the disease becomes manifest.[20,21] Such studies use blood samples collected historically from patients known at the time of the study to have RA. Since, for patients presenting with arthralgia, it is relevant to know absolute risks for development of arthritis, this review focused on longitudinal studies. Most cohort studies that investigated the presence of autoantibodies have studied seropositive (ACPA and/or RF) patients in clinically ill-defined groups; one cohort study evaluated patients with CSA (Table 2.1). In agreement with previous nested case-control studies, several longitudinal cohort studies have shown that the presence of ACPA associated with the development of clinical arthritis.[22–26] The value of the level of ACPA (within ACPA-positive patients) in predicting arthritis development is unclear. While two studies, reporting on the same cohort, found an association between ACPA level and arthritis development,[22,23] two other studies did not.[26,27] Although these three cohorts selected ACPA-positive patients with arthralgia using different inclusion criteria (seropositive arthralgia, CSA or ACPA-positive persons with non-specific MSK symptoms) in different settings (primary and/or secondary care), the contrasting results are not yet explained. In addition to ACPA level, other ACPA characteristics have also been studied. The number of epitopes recognised by ACPA was associated with arthritis development in several studies in ACPA-positive patients with arthralgia.[28–30] In addition, a decrease in galactosylation and an increase in core fucosylation of serum ACPA IgG1, indicating a change towards a more inflammatory phenotype of these autoantibodies, have been observed prior to the onset of RA.[31]

The value of RF in the preclinical phase of RA has also been studied.[22-24,26,32] Two studies, on the same cohort, performed stratified analyses and observed that within ACPA-positive patients, the additive presence of RF associated with arthritis development.[22,23] These studies did not contain ACPA-negative patients; hence, no information could be provided on the single presence of RF. Two studies, on the same cohort, did contain an RF-negative group and showed in univariable

analyses that the presence of RF conferred a higher risk of arthritis; however, after adjusting for the concomitant presence of ACPA, this association was lost.[24,26] Therefore it remains to be determined if the single presence of RF in arthralgia is a true predictor, although one study suggested that high levels of RF are a predictor in contrast to low levels of RF.[26]

Finally the presence of anti-carbamylated protein (anti-CarP) antibodies in the preclinical phase of RA was studied. One study in autoantibody-positive individuals observed an association between anti-CarP antibodies and the development of arthritis,[33] whereas another study in patients with CSA did not observe an additive value of anti-CarP antibodies when ACPA and RF status is known.[26]

In conclusion, the presence of ACPA is associated with arthritis development while this is less clear for RF and anti-CarP antibodies. A disadvantage of most current studies is that patients are selected based on autoantibodies; thus, there is no autoantibody-negative reference group. In addition, as inclusion of patients in these cohorts was driven largely by ACPA positivity, these patients would not necessarily have been defined as CSA and would not necessarily have fulfilled the EULAR definition of arthralgia. Furthermore as noted above, some of the available data are based on analyses of the same patient cohorts (studies in Table 2.1 reported on six cohorts). Finally, in clinical practice where patients present with arthralgia, it is important to estimate absolute risks for progression to arthritis, but many studies did not provide these risks. Studies that did determine positive predictive values (PPVs) observed that the PPV of ACPA (independent of RF) ranged between 16% and 50%.[22,26] This broad range can be explained by differences in patient settings, since PPVs are dependent on the prior risks of arthritis development, which varied in the different settings that were studied.

Table 2.1 Autoantibodies in the preclinical phase of RA

Author, Year	Cohort	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Measured factors	Main result
De Bois et al, 1996[32]	Arthralgia (secondary care)	52†	10 (21)	NP	12	Presence of IgM-RF	RF predicts development of RA; PPV 50%, NPV 100%.
Bos et al, 2010[22]	ACPA+ and/or RF+ arthralgia (secondary care)	147	29 (20)	11 (5-17)	28 (19-39)	Presence and level of IgM-RF and ACPA	Factors associated with arthritis development: - within all patients: ACPA (HR 6.0 95% CI 1.8-20), but not RF. - within ACPA+ patients: RF (HR 3.0 95% CI 1.4-6.9) and high ACPA levels (HR 1.7 95% CI 1.1-2.5). PPV for arthritis development within 2 years: ACPA-RF+ 6%, ACPA+RF- 16%, ACPA+RF+ 40%.
Van de Stadt et al, 2011[28]	ACPA+ and/or RF+ arthralgia (secondary care)	244	69 (28)	11 (5-20)	36 (18-60)	Reactivity of ACPA to 5 citrullinated peptides	Cox regression analysis within ACPA+ patients showed a trend between arthritis development and recognition of 2-5 peptides vs. 0-1 peptides (HR 1.7 95% CI 0.9-3.2).
Shi et al, 2013[33]	ACPA+ and/or RF+ arthralgia (secondary care)	340	129 (38)	12 (6-24)	36 (20-52)	Presence and level of anti-CarP IgG antibodies	Anti-CarP antibodies, but not anti-CarP levels, predicted progression to RA, independent of ACPA and RF (HR 1.6 95% CI 1.1-2.3). PPV for arthritis development: ACPA+anti-CarP- 40%, ACPA+anti-CarP+ 58%.
Van de Stadt et al, 2013[23]	ACPA+ and/or RF+ arthralgia (secondary care)	374	131 (35)	12 (6-23)	32 (13-48)	Presence and level of IgM-RF and ACPA	ACPA was associated with progression to arthritis when compared to RF+ACPA- patients: ACPAlow+RF- HR 2.7 95% CI 1.3-5.6, ACPAhigh+RF- HR 4.9 95% CI 2.5-9.6, ACPA+RF+ HR 6.9 95% CI 3.7-13.1.
De Hair et al, 2014[29]	ACPA+ and/or RF+ individuals at risk for RA (secondary care and public fairs)	55‡	15 (27)	13 (6-27)	24 (14-47)	Presence and level of IgG ACPA and reactivity to 10 citrullinated peptides	Total number of citrullinated peptides recognized by ACPA was associated with arthritis development (HR 1.2 95% CI 1.0-1.4). Proportion of ACPA+ patients and ACPA level were not different in patients with and without progression to arthritis.
Rakieh et al, 2015[27]	ACPA+ persons with aspecific musculoskeletal symptoms (primary and secondary care)	100	50 (50)	7.9 (0.1-52)	20 (0.1-69)	IgM-RF and ACPA levels	A measurement combining high level of RF and/or ACPA was not associated with arthritis development (HR 1.5, 95% CI 0.5-4.5, independent of tenderness of small joints, morning stiffness, PD signal and SE).

Table 2.1 continued

Author, Year	Cohort	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Measured factors	Main result
Rombouts et al, 2015[31]	ACPA+ arthralgia (secondary care)	183[§]	105 (57)	12 (6-24)	35 (21-52)	Fc glycosylation pattern of ACPA-IgG1 and total IgG1.	ACPA display decreased Fc galactosylation and increased fucosylation prior to the onset of RA.
Janssen et al, 2016[30]	ACPA+ and/or RF+ arthralgia (secondary care)	34	14 (41)	17 (5–35)	40 (24-43)	Total Ig-RF and IgG-ACPA levels and ACPA reactivity to 4 citrullinated peptides	Within those who developed RA, ACPA and RF levels were not increased at time of diagnosis compared with 6 months before diagnosis. Patients with progression to arthritis had a broader IgG ACPA repertoire and more IgA reactivity for Fib1.
Van Steenbergen et al, 2016[24]	Clinically suspect arthralgia (secondary care)	150[#]	30 (20)	1.7 (0.8-4.1)	17 (9-24)	IgM-RF and ACPA presence	In univariable analyses both ACPA and RF were associated with arthritis development (ACPA: HR 10 95% CI 4.9-21; RF: HR 6.9 95% CI 3.3-14). PPV for arthritis development within 1 year: ACPA 63%.
Nam et al, 2016 [25]	Persons with aspecific musculoskeletal symptoms (primary care)	2028	47 (2.3)	ACPA+ 1.8 (1.0-4.3) ACPA- 5.1 (2.9-14)	ACPA+ 12 (1.5-28) ACPA- 14 (13-22)	ACPA presence	RR for developing RA within 12 months in ACPA+ group was 67 (95% CI 32-138) and for IA it was 46 (95% CI 25-82). PPV of ACPA for development of RA was 42% and of IA 47%.
Ten Brinck et al, 2017 [26]	Clinically suspect arthralgia (secondary care)	241	44 (18)	3.6 (1.2-4.8)	103 (81-114)	IgM-RF, ACPA, anti-CarP antibody presence and ACPA and IgM-RF level	ACPA, RF and anti-CarP were associated with arthritis development but only ACPA was independently associated (HR 5.3 95% CI 2.0-14). RF levels but not ACPA levels were associated with progression to arthritis. PPV for arthritis development within 2 years: ACPA-RF+ 38%, ACPA+RF- 50%, ACPA+RF+ 67%.

Patients in refs 22,23,28,29,31,33 and in refs 24,26 are derived from the same cohort. Studies depicted in grey have provided absolute risks. [†]5 patients were lost to follow-up. In this study there was no correction for the presence of ACPA. [‡]IgM-RF and/or ACPA-positive individuals with arthralgia (n=34) or with a first degree relative with RA with or without arthralgia (n=16). Information on family history of RA was missing for 5 patients in whom no arthritis developed. [§]Patients in this study were selected based on high ACPA serum level (median 419 U/mL, IQR 131.0-1216.0). [#]1 patient who developed gout during follow-up was excluded from analyses. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CI, confidence interval; Fib1, fibrinogen; HR, hazard ratio; IA, inflammatory arthritis; IQR, interquartile range; NP, not provided; NPV, negative predictive value; PD, power Doppler; PPV, positive predictive value; RA, rheumatoid arthritis; RF, rheumatoid factor; RR relative risk.

The predictive accuracy of non-antibody serological markers in arthralgia

Various acute phase reactants, cytokines, chemokines and other systemic markers have been studied in the preclinical phase of RA (Table 2.2). Results of studies evaluating CRP and erythrocyte sedimentation rate (ESR) are conflicting. Some studies have identified an association between CRP or ESR and arthritis development,[24,31] while others have not.[22,27,30,34-36] The only study showing an association between CRP level at study entry and development of arthritis included patients with CSA and did not select on the presence of autoantibodies.[24] Studies that showed no predictive value of CRP were mostly conducted in autoantibody-positive arthralgia.[22,27,30,34-36] This could imply that CRP has a predictive value in autoantibody-negative patients in particular; further studies are needed to clarify this.

Other serological markers have been assessed. In one study, differences were observed in the lipid profile of patients with and without progression to arthritis. After correction for ACPA, a lower apolipoprotein A1 level was associated with arthritis development.[37] Another study evaluated 14-3-3η and showed that the PPV of 14-3-3η for arthritis development was 86%. However, when corrected for ACPA and RF, 14-3-3η did not predict onset of arthritis.[38] Other serological biomarkers showed trends towards higher levels in patients with progression to arthritis.[34,36] None of these markers was evaluated in other studies.

In conclusion, most results on serological markers of inflammation have not been validated in independent studies. Only CRP has been studied in several cohorts of patients with seropositive arthralgia and was shown to be of limited value.

Table 2.2 Serological markers in the preclinical phase of RA

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Measured factors	Main result
Proteins							
Bos et al, 2010[22]	ACPA+ and/or RF+ arthralgia (secondary care)	147	29 (20)	11 (5-17)	28 (19-39)	CRP levels	CRP levels were similar in patients with and without arthritis development (3.0, IQR 1.1-4.7 and 2.3, IQR 0.9-5.0, p=0.81, respectively).
Limper et al, 2012[36]	ACPA+ and/or RF+ arthralgia (secondary care)	137	35 (26)	11 (3.7-18)	21 (6-48)	hsCRP, PCT and SPLA2 levels and TNF-α, IL-6, IL-12p70, IL-10 and IFN-γ	Biomarker levels were not significantly different in patients with and without progression to arthritis during follow-up.
Van de Stadt et al, 2012[37]	ACPA+ and/or RF+ arthralgia (secondary care)	348	116 (33)	12 (6-23)	24 (14-49)	Total cholesterol, HDLc, LDLc, triglycerides, apoA1 and apoB.	After correction for ACPA only ApoA1 was predictive of arthritis development (HR 0.5, 95% CI 0.3-0.9). For HDLc, a trend was observed (HR 1st vs. 2nd and 3rd tertiles 0.7, 95% CI 0.5-1.0).
De Smit et al, 2014[54]	ACPA+ and/or RF+ arthralgia (secondary care)	289	94 (33)	12 (6-20)	30 (13-49)	IgA, IgG and IgM antibody levels against Porphyromonas gingivalis	Anti-P gingivalis antibody levels at baseline were not elevated in patients with progression to arthritis compared with patients without progression.
Rakieh et al, 2015[27]	ACPA+ persons with aspecific musculoskeletal symptoms (primary and secondary care)	100	50 (50)	7.9 (0.1-52)	20 (0.1-69)	hsCRP levels	CRP level at baseline was not associated with arthritis development (uncorrected HR 1.3 95% CI 0.7-2.4). PPV for arthritis development: 56%.
Rombouts et al, 2015[31]	ACPA+ arthralgia (secondary care)	183§	105 (57)	12 (6-24)	35 (21-52)	ESR	ESR was increased prior to the diagnosis of RA (arthralgia at baseline: median 15.0 mm/h (IQR 7.0–25); RA at diagnosis: 25 mm/h (IQR 19–33)).
Janssen et al, 2016[30]	ACPA+ and/or RF+ arthralgia (secondary care)	34	14 (41)	17 (5–35)	40 (24-43)	CRP levels and ESR	At study entry CRP levels and ESR were comparable between patients with and without progression to arthritis.
Van Steenbergen et al, 2016[24]	Clinically suspect arthralgia (secondary care)	150#	30 (20)	1.7 (0.8-4.1)	17 (9-24)	CRP levels	CRP level was associated with arthritis development, independent of other clinical factors and MRI-detected inflammation (HR 1.1 95% CI 1.0-1.1). PPV for arthritis development: 32%.

Table 2.2 continued

Proteins							
Beers-Tas et al, 2016[38]	ACPA+ and/or RF+ arthralgia (secondary care)	144	43 (30)	15 (0-60)	60 (1-60)	14-3-3η	14-3-3η was associated with arthritis development in patients with seropositive arthralgia, but when corrected for ACPA and RF 14-3-3η did not predict onset of arthritis. PPV of 14-3-3-η for arthritis development: 86%.
Chalan et al, 2016[34]	ACPA+ and/or RF+ arthralgia (secondary care)	27	11 (41)	8 (1-32)	Patients with non-progressing arthralgia: 26 (6-33)	25 serum immune markers: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, IFN-α , IFN-γ, GM-CSF, TNF-α, IL-1RA, IL-2 R, Eotaxin (CCL11), IL-8, IP-10 (CXCL10), MCP-1 (CCL2), MIG (CXCL9), MIP-1α (CCL3), MIP-1β (CCL4), Rantes (CCL5).	Trends for increase in IL-5, MIP-1β, IL-1RA and IL-12 in patients with arthralgia with progression to arthritis. AUC for IL-5 was 0.8 (95% CI 0.6-1.0). ESR and CRP were not significantly different in patients with and without progression to RA.
Zufferey et al, 2017[35]	RF- and ACPA-polyarthralgia of >6 weeks' duration (secondary care)	80	9 (11)	NP	18 (7)[†]	CRP levels	CRP level was not predictive of RA in univariable or multivariable regression analysis (OR 3.0 95% CI 0.4-24, corrected for gender and US score). PPV of CRP for development of arthritis: 22%.
PBMCs and expression of cell surface markers							
Janssen et al, 2016[30]	ACPA+ and/or RF+ arthralgia (secondary care)	34	14 (41)	17 (5–35)	40 (24-43)	Treg number and subsets.	Treg number and subsets were comparable in patients with and without progression to arthritis during follow-up.
Lübbers et al, 2015[51]	ACPA+ and/or RF+ arthralgia (secondary care)	155	44 (38)	8 (5-13)	23 (12-30)	B cell signature, comprising CD19, CD20, CD79α, CD79β.	Combination of low B cell score and high type I IFN signature predicts arthritis development in seropositive arthralgia. AUC for B cell score combined with ACPA and RF was 0.9 (95% CI 0.8-1.0) in IFN^{high} group and 0.7 (95% CI 0.6-0.8) in IFN^{low} group. PPV of $IFN^{high}Bcell^{low}$ score for development of arthritis: 60%.
Lübbers et al, 2016[50]	ACPA+ and/or RF+ arthralgia (secondary care)	113	40 (35)	13 (7.4-22)	27 (19-42)	Absolute number of $CD14^{+}$monocytes, $CD4^{+}$, $CD8^{+}$, $CD56^{+}$ T cells ($CD3^{+}$), $CD80^{+}$, $CXCR3^{+}$, $CD27^{+}$ B cells ($CD19^{+}$) and $CD16^{+}CD56^{+}CD3^{-}$ NK cells	Decreased CD8+ T cells and memory B cells in patients who developed arthritis.

Table 2.2 continued

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Measured factors	Main result
PBMCs and expression of cell surface markers							
Hunt et al, 2016[49]	ACPA+ persons with aspecific musculoskeletal symptoms (primary and secondary care)	103	48 (47)	63% progressed within 12 months	18 (0.1-80)[ǂ]	Naïve T cells, inflammation-related cells and regulatory T cells	T cell subset dysregulation in ACPA+ individuals predates the onset of inflammatory arthritis, predicts risk and faster progression to inflammatory arthritis. PPV for T cell subset combined with clinical factors was 60%. PPV of clinical model alone was 50%.
Gene expression total blood							
Van Baarsen et al, 2010[55]	ACPA+ and/or RF+ arthralgia (secondary care)	109	20 (18)	7 (4-15)	30 (22-39)	Gene expression profile	Signatures associated with arthritis development were involved in IFN-γ mediated immunity, hematopoiesis, and chemokine/ cytokine activity.
Limper et al, 2012[36]	ACPA+ and/or RF+ arthralgia (secondary care)	137	35 (26)	11 (3.7-18)	21 (6-48)	mRNA expression levels of 21 inflammatory genes	Biomarker levels were not significantly different in patients with and without progression to arthritis during follow-up.
Lübbers et al, 2013[56]	ACPA+ and/or RF+ arthralgia (secondary care)	115	44 (38)	8 (5-13)	23 (12-30)	Expression level of 7 type I IFN response genes: IFI44L, IFI6, IFIT1, MXA, OAS3, RSAD2, EPSTI	HR for development of arthritis was 2.4 (95% CI 1.3-4.5) for IFN^{high} individuals, corrected for ACPA and RF. AUC for IFN-score combined with ACPA and RF was 0.8 (95% CI 0.7-0.9). PPV of ACPA/RF combined with IFN score for development of arthritis: 65%.
Tak et al, 2017[52]	Seropositive individuals (ACPA and/or RF) at risk for RA	71	26 (37)	NP	Test cohort: no arthritis 69 (42-78), arthritis 15 (0-65). Validation cohort: at least 36 months.	Dominant BCR clones (BCR signals representing ≥0.5% of the repertoire) in PB and synovial tissue.	Presence of ≥5 dominant BCR clones in PB was associated with arthritis development (validation cohort RR 6.3, 95% CI 2.7-15). PPV of ≥5 dominant BCR clones for development of arthritis: 72% in test cohort and 83% in validation cohort.

Patients in refs 22,31,36,37,52,54,55, in refs 30,34, in refs 27,49 and in refs 38,50,51,56 are derived from the same cohort. Studies depicted in grey have provided absolute risks. [§]Patients in this study were selected based on high ACPA serum level (median 419U/mL, IQR 131.0–1216.0). [#]1 patient that developed gout during follow-up was excluded from analyses. [ǂ]mean (SD). [ǂ]median (range).

ACPA, anti-citrullinated protein antibodies; apo, apolipoprotein; AUC, area under curve; BCR, B cell receptor; CD, cluster of differentiation; CI, confidence interval; EPSTI, epithelial stromal interaction; ESR, erythrocyte sedimentation rate; GM-CSF, granulocyte macrophage colony-stimulating factor; HDLc, high-density lipoprotein cholesterol; HR, hazard ratio; (hs)CRP, (high sensitivity) C-reactive protein; IFN, interferon; IFI6, interferon alpha-inducible protein 6; IFI44L, interferon-induced protein 44 like; IFIT1, interferon induced protein with tetratricopeptide repeats 1; IL, interleukin; IQR, interquartile range; LDLc, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; MIG, monokine induced by gamma interferon; MIP, macrophage inflammatory protein; MRI, magnetic resonance imaging; MXA, myxovirus resistance protein A; NK cells, natural killer cells; NP, not provided; OAS3, 2'–5'-oligoadenylate synthetase 3; OR, odds ratio; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; PCT, procalcitonin; PPV, positive predictive value; RA, rheumatoid arthritis; RANTES, regulated on activation, normal T cell expressed and secreted; RF, rheumatoid factor; RR, relative risk; RSAD2, radical s-adenosyl methionine domain containing 2; SPLA2, secretory phospholipase A2; TNFα, tumor necrosis factor α; Treg, regulatory T cell; US, ultrasound.

The predictive accuracy of imaging markers detecting subclinical inflammation in arthralgia

Different imaging modalities (US, MRI, positron emission tomography and scintigraphy) have been used to study the presence of local subclinical inflammation[24,27,29,32,35,39-46]; most studies focused on US (Table 2.3) or MRI (Table 2.4).

Studies assessing the value of US have provided inconsistent results; some studies did not observe significant associations between US abnormalities and arthritis development,[27,43,44] while others did.[35,45,46] The studies that did not observe an association either included patients with seropositive arthralgia, ACPA-positive persons with non-specific MSK symptoms or patients with new-onset inflammatory arthralgia; studies that did observe an association included patients with arthralgia based on clinical characteristics, and differences in results might be partly explained by differences in patient selection. Furthermore, US protocols, joint regions assessed and US features reported on differed across the studies. It is also important to note that none of the studies have used a healthy reference population to define thresholds at which US findings should be classified as abnormal. Since a previous study has shown that US lesions (greyscale synovial effusion or synovitis with or without power Doppler signal) are also present in the majority (88%) of healthy volunteers, it might be important to correct for normal, physiological findings when defining a positive US.[47] Finally, few studies have evaluated the predictive value of US abnormalities in relation to the presence of other predictors; therefore, the additive value of US abnormalities to regularly used biomarkers is unknown. Despite these shortcomings, the data obtained suggest that of the different US features, power Doppler signal might have the

highest predictive value for the development of arthritis.[45,46]

Studies on the predictive value of MRI have been performed. Studies within autoantibody-positive non-specified arthralgia did not observe associations between MRI features at the knee (bone marrow edema (BME) or synovitis) and progression to clinical arthritis.[29,39] A small MRI study evaluating synovitis and BME in small joints of 28 patients with ACPA-positive arthralgia was also negative.[40] However, larger studies in 150 patients with CSA revealed that MRI-detected inflammation was associated with progression to arthritis, independent of ACPA, CRP and clinical factors.[24,41] Interestingly, in multivariable analyses, the effect size of MRI-detected inflammation was almost equal to that of ACPA (HR 5.1 for MRI and 6.4 for ACPA). MRI-detected tenosynovitis had a higher accuracy than synovitis or BME.[24] Altogether subclinical inflammation identified by MRI is a predictor for RA development, when measured in small hand and feet joints, but not in knee joints (which may not be the location where synovitis begins in RA). As with US, age-matched symptom-free controls to define thresholds at which MRI features should be viewed as abnormal are lacking in most MRI-based studies. This may have affected the results as it has been recently shown that the predictive accuracy and specificity of a positive MRI increased when this was taken into account.[48] Finally only one MRI study provided PPVs and observed that an abnormal MRI result (in patients with CSA) was associated with a risk for arthritis development during the next year of 31%.[24]

In conclusion, imaging studies in arthralgia have been conducted in different patient populations, evaluating different joints and different inflammatory features. None of the studies were independently replicated and none compared MRI and US in the same patients with arthralgia. Further studies using similar protocols in homogeneous patient groups are warranted.

Table 2.3 Ultrasonography in the preclinical phase of RA

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Locations scanned	Ultrasound/measured factors				Controls used to define positive US	Main result
							GS US	PD US	Teno-syno-vitis	Ero-sions		
Van de Stadt et al, 2010[43]	ACPA+ and/or RF+ arthralgia (secondary care)	192	45 (23)	11 (9)*	26 (6-54)	Only painful joints and adjacent and contralateral joints.	Y	Y	Y	N	N	At patient level US abnormalities were not associated with arthritis development.
Pratt et al, 2013[44]	Main study on arthritis, 46 patients with new-onset inflammatory arthralgia (secondary care)	379†	162 (42)	NP	28 (NP)	MCP, PIP and MTP joints, bilaterally	Y	Y	N	Y	N	The presence of MSUS abnormalities was not associated with development of persistent inflammatory arthritis in patients presenting with arthralgia, in the absence of clinical synovitis.
Rakieh et al, 2015[27]	ACPA+ persons with aspecific musculoskeletal symptoms (primary and secondary care)	100	50 (50)	7.9 (0.1-52)	20 (0.1-69)	Wrist, MCP and PIP joints, bilaterally	N	Y	N	N	N	PD signal was not associated with arthritis development (HR 1.9, 95% CI 0.8-4.2, independent of tenderness of small joints, morning stiffness, RF and/or ACPA and SE). PPV of PD signal for development of arthritis: 67%.
Van der Ven et al, 2016[45]	Arthralgia in ≥2 joints in hands, feet or shoulders <1 year (secondary care)	196‡	36 (23)	NP	NP (max. 12 months)	Wrist, MCP, PIP and MTP joints, bilaterally	Y	Y	N	N	N	The presence of PD signal (OR 5.8 95% CI 1.8–19) was associated with development of arthritis, independent of ACPA.
Nam et al, 2016[46]	ACPA+ persons with aspecific musculoskeletal symptoms (primary and secondary care)	136	57 (42)	8.6 (0.1-52)	18 (0.1-80)	Wrist, MCP, PIP and MTP joints, bilaterally	Y	Y	N	Y	N	Both GS and PD associated with arthritis development: GS ≥2 HR 2.8 (0.4-20), PD=2 HR 3.7 (2.0-6.9). PPV of GS ≥2 for development of arthritis was 48% and of PD=2 75%.

Table 2.3 continued

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	Locations scanned	Ultrasound/measured factors				Controls used to define positive US	Main result
							GS US	PD US	Teno-syno-vitis	Ero-sions		
Zufferey et al, 2017[35]	RF- and ACPA-polyarthralgia of >6 weeks duration (secondary care)	80	9 (11)	NP	18 (7)[§]	Wrist, MCP, PIP, elbow and knee joints, bilaterally	Y	N	N	N	N	US synovitis at baseline was associated with progression to RA. OR was 7.5 (95% CI 1.2-43) for SONAR >8/66 and 10 (95% CI 1.1-49) for grade ≥2 in ≥2 joints, independent of gender and CRP. PPV of US significant synovitis for development of arthritis: 25%.

Studies depicted in grey have provided absolute risks.
*mean (SD). [†]46/379 had a swollen joint count ≥1 at baseline. Outcome was persistent inflammatory arthritis. [‡]only 154 completed the 12 months follow-up. [§]mean (SD)
ACPA, anti-citrullinated protein antibodies; CI, confidence interval; CRP, C-reactive protein; GS, grey scale; HR, hazard ratio; IQR, interquartile range; MCP, metacarpophalangeal; MSUS, musculoskeletal ultrasound; MTP, metatarsophalangeal; N, no; NP, not provided; OR, odds ratio; PD, power Doppler; PIP, proximal interphalangeal; PPV, positive predictive value; RA, rheumatoid arthritis; RF, rheumatoid factor; SE, shared epitope; SONAR, Swiss sonography in arthritis and rheumatism; US, ultrasound; Y, yes.

Table 2.4 Magnetic resonance imaging in the preclinical phase of RA

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	MRI strength	Contrast enhance-ment	Locations scanned	Measured factors	Controls used to define positive MRI	Main result
Van de Sande et al, 2011[39]	ACPA+ and/or RF+ arthralgia (secondary care)	13*	4 (31)	3 (1-6)§	37 (25-45)§	1.5T	Y	Knee joint	Maximal enhancement, rate of enhancement, synovial volume and enhancement shape curve distribution	N	No differences in MRI findings between patients with and without progression to arthritis.
De Hair et al, 2014[29]	ACPA+ and/ or RF+ individuals at risk for RA (secondary care and public fairs)	55†	15 (27)	13 (6-27)	24 (14-47)	1.5T or 1T	Y	Arbitrary knee joint	Synovitis and hydrops in 4 compartments, BME, erosions and cartilage damage	N	None of the MRI parameters were associated with arthritis development.
Gent et al, 2014[40]	ACPA+ arthralgia (secondary care)	28	12 (43)	NP	NP 3 years follow-up	1.5T	Y	Wrist, MCP and PIP joints of both hands	Synovitis and BME according to RAMRIS	N	No difference in MRI-detected synovitis and BME scores in patients with and without progression to arthritis.
Van Steenber-gen et al, 2014[41]	ACPA- clinically suspect arthralgia (secondary care)	64	5 (8)	NP	9 (5-11)	1.5T	Y	Wrist, MCP and MTP joints, of most painful side	Synovitis and BME according to RAMRIS	N	Higher scores for MRI-inflammation (sum of BME and synovitis scores), synovitis and BME in patients who developed clinically detectable arthritis.

Table 2.4 continued

Author, Year	Study population	Cases (n)	Progression to arthritis (%)	Median duration from study entry to diagnosis of arthritis, months (IQR)	Median duration of follow-up, months (IQR)	MRI strength	Contrast enhance-ment	Locations scanned	Measured factors	Controls used to define positive MRI	Main result
Van Steenber-gen et al, 2016[24]	Clinically suspect arthralgia (secondary care)	150[‡]	30 (20)	1.7 (1-4)	17 (9-24)	1.5T	Y	Wrist, MCP and MTP joints, of most painful side	Synovitis and BME according to RAMRIS. Tenosynovitis in wrist and MCP joints.	Y	MRI-detected inflammation was associated with progression to arthritis, independent of age, symptom localisation, CRP and ACPA (HR 5.1, 95% CI 1.8-15). PPV of MRI-detected inflammation for arthritis development within 1 year: in all patients 31%, in ACPA+ patients 71%.

Patients in refs 25,35,37 are all recruited via referral from the Academic Medical Center, Amsterdam, and from the rheumatology outpatient clinic of Reade. Patient in refs 19,38,39 are all included in the Leiden Clinically Suspect Arthralgia cohort. The study depicted in grey has provided absolute risks.
*IgM-RF and/or ACPA-positive individuals with arthralgia (n=12) or with a first degree relative with RA with arthralgia (n=1).
[§]Median (range).
[†]IgM-RF-positive and/or ACPA-positive individuals with arthralgia (n=34) or with a first-degree relative with RA with or without arthralgia (n=16). Information on family history of RA was missing for five patients in whom no arthritis developed.
[‡]One patient who developed gout during follow-up was excluded from analyses. In six patients MRI was not performed.
ACPA, anti-citrullinated protein antibodies; CI, confidence interval; CRP, C-reactive protein; BME, bone marrow edema; HR, hazard ratio; IQR, interquartile range; MCP, metacarpophalangeal; MRI, magnetic resonance imaging; MTP, metatarsophalangeal; N, no; NP, not provided; PIP, proximal interphalangeal; PPV, positive predictive value; RA, rheumatoid arthritis; RAMRIS, rheumatoid arthritis MRI scoring system; RF, rheumatoid factor; Y, yes.

Markers characterising immune cell dysfunction

It has been suggested that immune system dysregulation is an early feature of RA frequently preceding the onset of arthritis. Several markers have been studied. The number of regulatory T cells (Tregs) in the peripheral blood appeared not to be indicative of RA development in patients with seropositive arthralgia.[30] In contrast, others showed that reduced naïve T cells and Tregs and increased inflammation-related cells were predictive of progression to arthritis in ACPA-positive persons with non-specific MSK symptoms.[49] Seropositive patients who developed arthritis had a significantly decreased number of peripheral CD8+ T cells and memory B cells compared with non-converters.[50] B cell subtypes have been studied; patients with seropositive arthralgia with a low B cell score, measured as expression of CD19, CD20, CD79α and CD79β, had an increased risk of arthritis if there was also a high type I interferon signature.[51] B cell receptor (BCR) clones, defined as BCR clones expanded beyond 0.5% of the total repertoire, have also been studied in the peripheral blood of 71 seropositive individuals at risk of RA and were associated with an enhanced risk of arthritis.[52]

Unfortunately, most of the abovementioned studies did not address whether the novel markers added to the predictive utility of regularly used biomarkers and validation was lacking. In addition, most of the studied markers are not high-throughput available in daily clinical practice.

Conclusion

The processes causing arthralgia to progress to clinically evident RA are insufficiently understood. Most studied predictors are not pathognomonic for this transition or for RA, and the predictive accuracy of most markers has not been validated in different studies. Only ACPA positivity has been observed to associate with RA development across multiple studies. In addition none of the predictors studied, including ACPA, was sufficiently predictive on its own, and the vast majority of studies did not combine different types of predictors. The few studies that did combine different markers (e.g., imaging and ACPA) revealed that combinations were also insufficient for adequate risk stratification in many patients (as PPVs were <80%).[24] Therefore more research is needed to obtain adequate risk stratification in patients with arthralgia.

Ideally, future studies should be performed in homogeneous patient groups, for example, patients fulfilling the EULAR definition of arthralgia at risk for RA. In this way, patients with comparable prior risks for RA will be selected, and validation of findings in different cohorts will be possible. Results of these future studies should provide data to support the development of robust algorithms to differentiate patients with arthralgia likely to progress to RA from those unlikely to do so. Importantly the variables within these algorithms and their weightings may well be different for algorithms designed for use in different contexts, for example, primary and secondary care.

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3

The 2010 ACR/EULAR criteria are not sufficiently accurate in the early identification of autoantibody-negative rheumatoid arthritis: Results from the Leiden-EAC and ESPOIR cohorts

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Abstract

Objectives

The 2010 ACR/EULAR criteria were derived to classify rheumatoid arthritis (RA) earlier in time. Previous studies indeed observed that the 2010 criteria were fulfilled earlier than the 1987 criteria. This study determined whether the 2010 criteria perform equally in early classification of autoantibody-positive and autoantibody-negative RA.

Methods

From the total Leiden EAC (n=3448) and ESPOIR (n=813) RA patients who fulfilled the 1987 RA criteria at 1 year but not at presentation were selected (n=463 and n=53, respectively), as these patients were classified with delay with the 1987 criteria. These RA patients were studied on fulfilling the 2010 criteria at baseline (as 2010 positivity indicated that these RA patients were earlier identified) and these analyses were stratified for patients with and without anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). Analyses were repeated for disease-modifying antirheumatic drug (DMARD) start within the first year as reference for RA (instead of fulfilling the 1987 criteria).

Results

In the EAC, 75% of the selected RA patients did already fulfil the 2010 criteria at baseline. In ESPOIR this was 57%, indeed demonstrating early classification with the 2010 criteria. Among the selected autoantibody-positive RA patients of the EAC, 93% was already identified at baseline with the 2010 criteria. Within autoantibody-negative RA this was 51% ($p<0.001$), indicating that 49% of autoantibody-negative RA patients were not early classified with the 2010 criteria. Similarly, within autoantibody-positive RA patients in ESPOIR 92% were 2010 positive at baseline, whereas this was only 25% within autoantibody-negative RA ($p<0.001$), indicating that 75% of autoantibody-negative RA patients were not early classified with the 2010 criteria. Similar results were obtained when DMARD start was the reference for RA.

Conclusions

The 2010 criteria perform well in the early identification of autoantibody-positive RA, but autoantibody-negative RA patients are still frequently missed with these criteria. This implies that other diagnostics are required for ACPA-negative patients.

Introduction

Previously, the 1987 ACR criteria were used to classify patients as rheumatoid arthritis (RA).[1] Because these criteria had a low sensitivity to classify RA in an early stage, the 2010 ACR/EULAR criteria have been developed.[2] Several studies have indeed shown that the 2010 criteria are fulfilled earlier in time than the 1987 criteria.[3-5] Cader et al. nicely demonstrated that the 2010 criteria allowed earlier identification of RA than the 1987 criteria. Van der Linden et al. studied 2258 patients that did fulfil the 1987 criteria during the first year but not at baseline. A total of 68% of these patients did fulfil the 2010 criteria at baseline, thus these data also indicated that the 2010 criteria identified RA patients in an earlier phase of the disease.

Early identification of RA is relevant as it allows early treatment. Anti-citrullinated protein antibody (ACPA)-positive and ACPA-negative RA have different genetic and environmental risk factors and are considered as separate disease subsets. However, early treatment is associated with a higher chance on achieving disease-modifying antirheumatic drug (DMARD)-free sustained remission in both subsets.[6,7] Thus both ACPA-positive and ACPA-negative RA patients should be identified early. As described above several studies have been published on the performance of the 2010 criteria in the earlier identification of RA.[3-5,8,9] However, it is undetermined if the 2010 criteria perform equally well in the earlier identification of ACPA-positive RA and ACPA-negative RA, with the 1987 criteria as reference. The recent findings that ACPA-negative patients with RA according to the 2010 criteria have more inflammation than ACPA-positive patients with 2010-RA suggests that the 2010 criteria may perform differently in the early identification of ACPA-positive and ACPA-negative RA.[10,11] To investigate this, this study compared the earlier identification of RA by the 2010 criteria, in autoantibody-positive patients with that in autoantibody-negative patients using data of two early arthritis cohorts.

Materials and methods

Patients

Patients from two different cohorts were studied.[12,13] The Leiden Early Arthritis Clinic (EAC) is an inception cohort that started in 1993 and includes patients with clinically confirmed arthritis and symptom duration <2 years at presentation at the rheumatologist. The patients studied were included between 1993 and 2015.

The total EAC comprised 3448 patients with early arthritis; of these patients, 1645 were not classified as having RA (according to the 1987 criteria) or with other diagnoses at baseline (thus these patients had undifferentiated arthritis, UA) (Figure 3.1). The remaining 1803 patients had RA according to the 1987 criteria (n=751) or other diagnoses (n=1052) and were excluded from further analyses. The Evaluation et Suivi de POlyarthrites Indifférenciées Récentes (ESPOIR) is a cohort in which patients from 14 regional centers were recruited; it was started in 2002. Included were patients with a symptom duration ≤6 months and a high clinical suspicion on RA according to the rheumatologist. Patients were aged between 18 and 70 years and had ≥2 swollen joints for at least 6 weeks. The ESPOIR cohort comprised 813 patients with early RA or UA, included between 2002 and 2005. Of these patients 234 were classified as having UA and were included in the present study. The remaining 579 patients had RA according to the 1987 criteria and were excluded from further analyses. Both studies were approved by the local ethical committees; all patients signed informed consent.

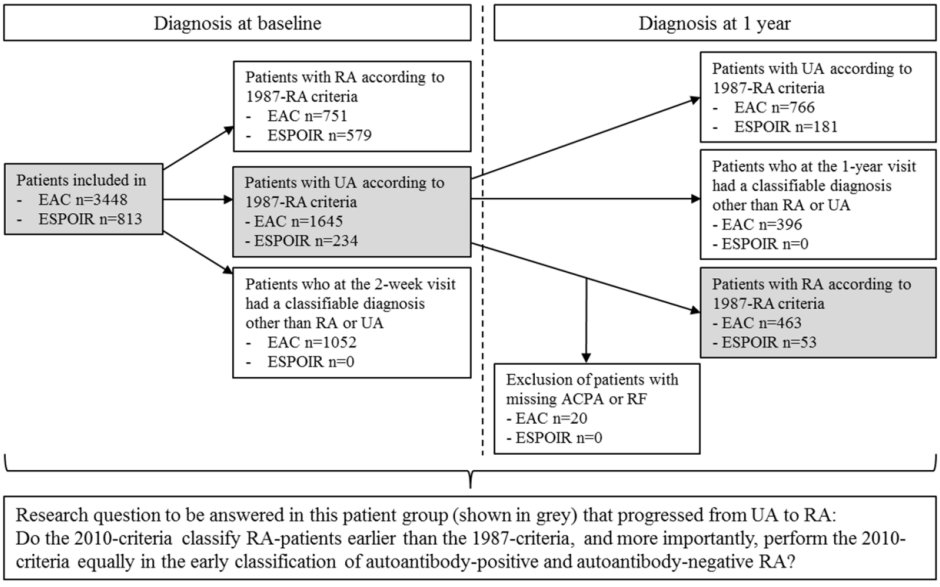

Figure 3.1 Flowchart of patient selection (patient group indicated in grey was studied) and research question

Patients that at the 2-week visit (when results of laboratory tests and radiographs were known) had clear diagnoses other than RA were excluded. Also RA patients who fulfilled the 1987 criteria at baseline were excluded. Patients that were classified as having UA according to the 1987 criteria were selected. Of these patients we selected patients who fulfilled the 1987 criteria after 1 year of follow-up; patients with other diagnoses were excluded from further analyses. The selected RA patients were identified with delay with the 1987 criteria. Of these patients it was determined whether they already fulfilled the 2010 criteria at baseline. RA, rheumatoid arthritis; UA, undifferentiated arthritis.

Analyses

The 1987 ACR criteria and 2010 ACR/EULAR criteria were applied as described.[1,2] Because up to 2010, the 1987 criteria were the reference for RA, fulfilling these criteria <1 year was used as reference for RA. In line with van der Linden et al.,[5] we selected RA patients that fulfilled the 1987 criteria within 1 year but not at baseline. These RA patients were identified with delay with the 1987 criteria. Of these patients it was determined whether they already fulfilled the 2010 criteria at baseline and thus were earlier recognized as having RA with the 2010 criteria. These earlier recognized RA patients were studied on the presence of ACPA and rheumatoid factor (RF). In subanalyses, DMARD initiation during the first year was used as reference for RA. The presence of ACPA and RF was determined with ELISA (EAC: RF, in-house ELISA[14] and anti-CCP2, eurodiagnostica, the Netherlands, cut-off value ≥25 U/ml and anti-CCP2, EliA CCP, Phadia, Nieuwegein, the Netherlands, cut-off ≥7 U/ml; ESPOIR: RF, Ménarini, France, cut-off value ≥9 U/ml and anti-CCP2, Diasorin, France, cut-off value ≥50 U/ml). Differences were tested with chi-square test or Fisher's exact test as appropriate. P-values <0.05 were considered significant. Analyses were performed using SPSS version 23.0 (IBM).

Results

Of the total Leiden-EAC, 1645 patients were diagnosed with UA at baseline according to the 1987 criteria (Figure 3.1). Of these RA patients, 483 did fulfil the 1987 criteria at 1 year. In total, 20 of these 483 patients were excluded from further analyses because of missing ACPA or RF, leaving 463 patients to study. Thus during the follow-up, 463 patients were diagnosed with RA; however, these patients were missed when applying the 1987 criteria at baseline. Within the ESPOIR cohort, 234 patients out of 813 RA and UA patients in total were diagnosed with UA at baseline according to the 1987 criteria. Out of these 234 patients 53 did fulfil the 1987 criteria at 1 year and thus were initially missed. Baseline characteristics of the patients studied are shown in Table 3.1.

Table 3.1 Baseline characteristics of patients fulfilling the 1987 RA criteria at 1 year but not at baseline

	Leiden EAC (n=463)		ESPOIR (n=53)	
	ACPA+ and/or RF+ (n=258)	ACPA- RF- (n=205)	ACPA+ and/or RF+ (n=25)	ACPA- RF- (n=28)
Age, mean (SD)	53 (15)	59 (16)	46 (10)	47 (14)
Female, n (%)	181 (70)	128 (62)	20 (80)	23 (82)
Symptom duration, median (IQR), weeks	20 (28)	17 (22)	26 (33)	20 (23)
TJC, median (IQR)	7 (11)	9 (13)	3 (3)	6 (7)
SJC, median (IQR)	5 (6)	7 (11)	3 (1)	4 (3)
ESR (mg/l), median (IQR)	25 (32)	29 (34)	16 (20)	15 (23)
CRP (mg/L), median (IQR)	10 (18)	15 (38)	6 (12)	2 (12)

ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; Leiden EAC, Leiden Early Arthritis Clinic; N, number of patients; RF, rheumatoid factor; SD, standard deviation; SJC, swollen joint count; symptom duration, time between symptom onset and inclusion in cohort; TJC, tender joint count.

Leiden EAC

When applying the 2010 criteria on the 463 RA patients at baseline, 75% (345/463) was identified as RA already at baseline according to the 2010 criteria (Table 3.2). In total, 200 patients (43%) were ACPA-positive and 263 patients (57%) were ACPA-negative. Of all 200 ACPA-positive RA patients missed at baseline with the 1987 criteria, 94% (188/200) was 2010 criteria positive at baseline. In contrast, only 60% (157/263) of the ACPA-negative RA patients was 2010 criteria positive at baseline ($p<0.001$). Similar analyses were performed when considering RF in addition to ACPA (Table 3.2). Of the 463 patients missed when applying the 1987 criteria at baseline, 258 patients (56%) were autoantibody-positive and 205 patients (44%) were autoantibody-negative. Of the 258 autoantibody-positive RA patients, who were missed at baseline with the 1987 criteria, 93% (240/258) did fulfil the 2010 criteria at baseline. In contrast, only 51% (105/205) of the autoantibody-negative RA patients was identified at baseline when applying the 2010 criteria ($p<0.001$).

Finally, to evaluate the frequency with which RA patients were not classified early in time with the current sets of criteria, the proportion of RA patients (fulfilling the 1987 criteria at 1 year) that was missed by both the 1987 and 2010 criteria at baseline was studied. Of the total Leiden-EAC, 1142 patients out of 2396 RA and UA patients in total were diagnosed with RA at 1 year according to the 1987 criteria. In all, 18 autoantibody-positive RA patients were missed at baseline by both the 1987 and 2010 criteria, which is 1.6% (18/1142) of the total number of RA patients. This

is in contrast to 100 autoantibody-negative RA patients that were missed by both criteria at baseline; this comprised 8.8% (100/1142) of the total RA patients.

ESPOIR

Of the 53 RA patients, 57% (30/53) was recognized earlier with the 2010 criteria (Table 3.2). In total, 15 patients (28%) were ACPA-positive and 38 patients (73%) were ACPA-negative. Of all ACPA-positive RA patients missed at baseline, 100% (15/15) was 2010 criteria positive at baseline, but only 39% (15/38) of the ACPA-negative RA patients was 2010 criteria positive at baseline (p<0.001). When also considering RF, 92% (23/25) of the autoantibody-positive RA patients was 2010 criteria positive at baseline, in contrast to 25% (7/28) of the autoantibody-negative RA patients (p<0.001).

Table 3.2 Proportion of antibody-positive and -negative patients earlier classified with the 2010 RA criteria (2010-RA baseline positive)

	Leiden EAC				ESPOIR			
	ACPA+	**ACPA-**	**Total**	**p-value**	**ACPA+**	**ACPA-**	**Total**	**p-value**
2010-RA baseline negative	12/200 (6%)	106/263 (40%)	118	<0.001	0/15 (0%)	23/38 (61%)	23	<0.001
2010-RA baseline positive	188/200 (94%)	157/263 (60%)	345		15/15 (100%)	15/38 (39%)	30	
	200	263	463		15	38	53	
	ACPA+ and/or RF+	**ACPA-RF-**	**Total**	**p-value**	**ACPA+ and/or RF+**	**ACPA-RF-**	**Total**	**p-value**
2010-RA baseline negative	18/282 (7%)	100/233 (49%)	170	<0.001	2/25 (8%)	21/28 (75%)	23	<0.001
2010-RA baseline positive	240/282 (93%)	105/233 (51%)	345		23/25 (92%)	7/28 (25%)	30	
	258	205	463		25	28	53	

Presented are the patients that fulfilled the 1987 RA criteria within 1 year but not at baseline. Of these patients the number of patients fulfilling the 2010 RA criteria at baseline is presented, divided into ACPA-positive and ACPA-negative patients and into autoantibody-positive and autoantibody-negative patients, respectively. ACPA: anti-citrullinated protein antibodies; RF: rheumatoid factor. Differences were tested with chi-square test or Fisher's exact test as appropriate.

Subanalyses

To ascertain the validity of our results, the use of DMARDs was also used as outcome measure instead of fulfilment of the 1987 RA criteria at 1 year; this revealed similar results (Table 3.3).

Table 3.3 Proportion of autoantibody-positive and -negative patients earlier classified with the 2010 RA criteria (outcome DMARD use)

	Leiden EAC				ESPOIR			
	ACPA+	**ACPA-**	**Total**	**p-value**	**ACPA+**	**ACPA-**	**Total**	**p-value**
2010-RA baseline negative	22/215 (10%)	224/421 (53%)	246	<0.001	0/34 (0%)	48/101 (48%)	48	<0.001
2010-RA baseline positive	193/215 (90%)	197/421 (47%)	390		34/34 (100%)	53/101 (52%)	87	
	215	421	636		34	101	135	
	ACPA+ and/or RF+	**ACPA-RF-**	**Total**	**p-value**	**ACPA+ and/or RF+**	**ACPA-RF-**	**Total**	**p-value**
2010-RA baseline negative	36/300 (12%)	217/360 (60%)	253	<0.001	3/52 (6%)	45/83 (54%)	48	<0.001
2010-RA baseline positive	264/300 (88%)	143/360 (40%)	407		49/52 (94%)	38/83 (46%)	87	
	300	360	660		52	83	135	

Instead of fulfilling the 1987 criteria at 1 year as outcome, DMARD initiation was used as outcome measure. Total numbers are patients not fulfilling the 1987 criteria at baseline but who are treated with DMARDs at 1 year follow-up. Of these patients the number of patients fulfilling the 2010 RA criteria at baseline is shown, divided into ACPA-positive and ACPA-negative patients and into autoantibody-positive and autoantibody-negative patients, respectively. ACPA, anti-citrullinated protein antibodies; RF, rheumatoid factor; DMARDs, disease-modifying antirheumatic drugs. Differences were tested with chi-square test or Fisher's exact test as appropriate.

Discussion

Studying patients from two early arthritis cohorts revealed that the 2010 criteria identify RA patients earlier than the 1987 criteria, which is in line with previous studies.[3-5] The present data now adds the information that autoantibody-positive RA in particular is earlier identified with the 2010 criteria, in contrast to autoantibody-negative RA.

This finding is not surprising since the autoantibodies ACPA and RF are heavily weighted in the 2010 criteria. Autoantibody-negative patients should have more than 10 tender or swollen joints together with abnormal acute phase reactants and ≥6 weeks symptom duration to be classified with RA. In contrast, autoantibody-positive patients can already fulfil the 2010 criteria when they only have two involved joints if they fulfil the acute phase reactants and symptom duration criteria.[2] Nordberg recently demonstrated that ACPA-negative patients fulfilling the 2010 criteria for RA indeed had more severe inflammation than ACPA-positive patients fulfilling these criteria.[10] The current data show a consequence of this recent finding for the early identification of RA. This finding is a consequence of the circularity of the different components of the criteria, and awarding a stronger weight on autoantibodies automatically implies that patients without

autoantibodies require more of the other components to fulfil the criteria. A recent meta-analysis on the 2010 criteria revealed that the 2010 criteria have a moderate specificity, especially when the expert opinion was used as reference.[15] Additionally, it has been observed that the long-term outcome of patients fulfilling the 2010 criteria is different from that of patients fulfilling the 1987 criteria, suggesting that the criteria identify a slightly different set of patients.[16] Despite the existing literature on the 2010 criteria, the consequence for the early classification of ACPA-negative RA in particular has not yet been clearly described.

Although the findings done here are a logic consequence of the composition of the 2010 criteria, the difference between the "old" versus the "new" classification criteria for RA can have consequences, for instance when the criteria are used to select patients for trials that are performed in very early phases of RA. When conducting clinical trials in early disease stages and fulfilment of the 2010 classification criteria is used in the inclusion criteria, autoantibody-positive patients in particular can be included in an early phase, in contrast to autoantibody-negative patients, who are less often classified as RA in an early phase.[8,17] Then future trials will reveal less evidence on the effect of treatments in early autoantibody-negative RA.

The 2010 criteria were developed for classification and not for diagnosis, but in practice may sometimes be used in the diagnostic process or influence the diagnostic process in daily practice. When this happens, ACPA-negative patients are possibly more often identified later in time than ACPA-positive patients. This is unfortunate as early treatment is observed to be relevant for both subsets of RA[6,7,18] Thus additional tools are required to also recognize these ACPA-negative RA patients early. In total, 10% of all RA patients are not early identified of which almost 9% are autoantibody-negative.

The pathogenesis of ACPA-negative RA is less well understood and presumably ACPA-negative RA consists of a variety of subgroups with differences in etiopathology. This latter view is supported by the finding that part of the ACPA-negative patients have no joint destruction, whereas others do have a severely destructive disease.[19] Despite the difficulties with the conception of ACPA-negative RA, it is nowadays questionable if ACPA-negative RA patients suffer less than ACPA-positive RA patients. Clinically relevant joint damage has become infrequent and several studies evaluated other disease outcomes and observed that ACPA-negative RA patients have at least as much functional disability and similar disease activity scores as ACPA-positive patients.[10,11,20] In addition, most ACPA-negative RA

patients do have a chronic disease course.[11] Moreover, it has been shown that early DMARD initiation is beneficial in ACPA-negative RA.[21] Finally, the recent findings that the majority of autoantibody-negative RA patients who do not fulfil the 2010 criteria do require DMARD therapy over time and have a persistent disease course underline the importance to also classify these RA patients early in time.[3,22] Based on the combination of these findings, we feel that early classification or early identification of ACPA-negative RA is relevant.

In this study, fulfilment of the 1987 RA criteria after 1 year follow-up was chosen as reference because these criteria perform well in advanced disease. Additionally, the 1987 criteria reflect the situation before the introduction of the new 2010 criteria which makes it a specific reference of RA. In sub-analyses, DMARD use during the first year was used as reference for RA and this showed similar findings for the performance of the 2010 criteria in the early classification of RA, showing robustness of the data.

A potential limitation is that the inclusion criteria of both cohorts were slightly different. The EAC is an inception cohort and the only referral center in a region. ESPOIR is a nationwide observational cohort of patients with suspected RA that not necessarily included all patients with RA in the participating regions. Furthermore, in contrast to ESPOIR, the EAC included patients with ≥1 swollen joint (and the RA criteria were applied in retrospect). These differences may explain the difference in proportion of RA patients who initially presented with UA. Despite these differences the trend in the data was similar.

Conclusions

In conclusion, this study showed that autoantibody-positive RA is more often early identified with the 2010 criteria than autoantibody-negative RA. This implies that other diagnostic methods or other diagnostic tests are required for the early identification and early classification of autoantibody-negative RA.

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4

Does information on novel identified autoantibodies contribute to predicting the progression from undifferentiated arthritis to rheumatoid arthritis? A study on anti-CarP antibodies as example

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Abstract

Background

The presence of autoantibodies is considered an important characteristic of rheumatoid arthritis (RA); therefore, both anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF) are included in the 2010 classification criteria for RA. However, a considerable number of RA patients lack both these autoantibodies. Recently, several novel autoantibodies have been identified but their value for the classification of RA patients is unclear. Therefore, we studied the value of novel autoantibodies using the presence of anti-carbamylated protein (anti-CarP) antibodies as an example for predicting RA development in patients with undifferentiated arthritis (UA).

Methods

There were 1352 UA patients included in the Leiden Early Arthritis Clinic cohort according to the 1987 criteria. When the 2010 criteria were used, there were 838 UA patients. Of these, we evaluated whether they fulfilled the 1987 or 2010 criteria after 1 year, respectively. Logistic regression analyses were performed with RA as outcome and ACPA, RF, and anti-CarP antibodies as predictors. Analyses were repeated after stratification for ACPA and RF.

Results

Thirty-three percent of the 1987-UA patients and 6% of the 2010-UA patients progressed to RA during the first year of follow-up. For the 1987-UA patients, anti-CarP antibodies were associated with progression to RA, an association which remained when a correction was made for the presence of ACPA and RF (OR 1.7, 95% CI 1.2-2.4). After stratification for ACPA and RF, anti-CarP antibodies were associated with progression to RA only for ACPA- and RF-negative patients (OR 2.1, 95% CI 1.3-3.7). For the 2010-UA patients, anti-CarP antibodies were associated with progression to RA; however, they were not when a correction was made for the presence of ACPA and RF (OR 0.8, 95% CI 0.3-2.1).

Conclusions

Our finding that anti-CarP antibodies have no additional value when RA is defined according to the 2010 criteria might be inherent to the composition of the 2010 criteria and therefore might also apply to other novel autoantibodies. Potentially it would be interesting to evaluate other, non-autoantibody biomarkers.

Background

Rheumatoid arthritis (RA) is characterized by the presence of autoantibodies, the most characteristic among which are anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). These are used as diagnostic tools and are included in the classification criteria for RA.[1] Nonetheless, in approximately one-third of early RA patients these autoantibodies are lacking.[2] It is important to better characterize these patients since early intervention in seronegative RA is also important. Therefore, research has focused on identifying novel autoantibodies and several have been identified.[3-7] Based on this research, two issues have been raised. First, stratified analyses are pivotal to prove an additive value of a test. A novel autoantibody should predict an outcome in patients negative for both ACPA and RF, or in patient groups with a similar presence of ACPA and/or RF (e.g., ACPA+RF+novel autoantibody+ vs. ACPA+RF+novel autoantibody- patients). Thus far, studies that have evaluated the predictive value of novel autoantibodies are often stratified for ACPA but not for RF, leaving the question unanswered if the findings attributed to the novel autoantibody were actually driven by the concomitant presence of RF.[5,8] A second issue is that, although different disease stages of RA have been studied, the value of novel autoantibodies in identifying the patients that will develop RA among patients presenting with undifferentiated arthritis (UA) is undetermined. Only one study evaluated the role of novel autoantibodies (UH-RA.1, UH-RA. 21) in UA patients as an early marker of RA development.[4] The ultimate aim of this study was to increase our understanding on the value of recently identified autoantibodies to predict RA development using accurate stratification for ACPA and RF. An interesting novel family of autoantibodies are the anti-carbamylated protein (anti-CarP) antibodies which target proteins modified by carbamylation. These antibodies are present in RA patients and are associated with the severity of radiographic progression.[7,9] In this study, we investigated the value of the novel anti-CarP antibodies in predicting RA development in patients with UA, independent of ACPA and RF.[7]

Methods

Patients

Between 1993 and 2015, 1352 UA patients (according to the 1987 criteria; 1987-UA) were included in the Leiden Early Arthritis Clinic (EAC) cohort. This became 838 UA patients when the 2010 criteria were used (2010-UA). The EAC is an inception cohort that was started in 1993 and includes patients with clinically confirmed

arthritis with symptom duration <2 years at presentation to the rheumatologist.[10] Baseline questionnaires, joint counts, and blood samples were collected, and radiographs were taken. Two weeks after inclusion, when the results of laboratory investigations and radiography were known, patients received their diagnosis. Classification criteria were only applied to patients with a clinical diagnosis or suspicion of RA, and patients who were not classified according to RA classification criteria were documented as having UA.

Anti-CCP2, RF and anti-CarP antibody measurements

Baseline serum samples were tested for ACPA, RF and anti-CarP antibodies. IgG antibodies to cyclic citrullinated peptide (CCP) were measured by second generation anti-CCP2 enzyme-linked immunosorbent assay (ELISA; Immunoscan RA Mark 2, Eurodiagnostica, Arnhem; cut-off 25 U/ml), and anti-CCP2 ELISA (EliA CCP, Phadia, Nieuwegein, the Netherlands; cut-off 7 U/ml). IgM RF was determined by an in-house ELISA. IgG anti-CarP antibodies were determined as described previously in the Leiden EAC.[7] As no commercial kit is available for anti-CarP antibodies, we used our own in-house anti-CarP assay based on carbamylated fetal calf serum and, as a control, nonmodified fetal calf serum as the coating antigens in the ELISA. Cut-off for positivity was based on the mean +2 standard deviations (SDs) from a set of healthy controls.

Analyses

Analyses were first performed when RA was classified using the 1987 criteria; thereafter, analyses were repeated using the 2010 criteria since autoantibodies are more prominent in the 2010 criteria. Fulfilment of the 1987 criteria and 2010 criteria was evaluated after 1 year of follow-up for the 1987-UA and 2010-UA patients, respectively. Logistic regression analyses were performed with ACPA, RF, and anti-CarP antibodies as independent variables and RA as outcome, both in the total group of UA patients and after stratification for ACPA and RF status.

Results

Baseline characteristics of the 1352 1987-UA and 838 2010-UA patients are shown in Table 4.1. Of these UA patients, 33% (441/1352) and 6% (53/838) progressed to RA during the first year according to the 1987 and 2010 criteria, respectively. Of the 1352 1987-UA patients, 257 (19%) were anti-CarP-positive and of the 838 2010-UA patients, 77 (9%) were anti-CarP-positive.

Table 4.1 Baseline characteristics of the total group of UA patients and the subgroups of patients with UA according to the 1987 and the 2010 criteria

	Total group of UA patients (n=1430)	Subgroup of 1987-UA patients (n=1352)	Subgroup of 2010-UA patients (n=838)
Age (years), mean (SD)	53 (17)	53 (17)	51 (17)
Female, n (%)	882 (62)	837 (62)	494 (59)
Symptom duration (weeks), median (IQR)	14 (6-31)	14 (6-31)	12 (5-28)
66-SJC, median (IQR)	3 (1-7)	3 (1-7)	2 (1-4)
68-TJC, median (IQR)	4 (1-10)	4 (1-10)	2 (1-5)
CRP (mg/ml), median (IQR)	8 (3-22)	8 (3-22)	6 (3-19)
ACPA positivity, n (%)	297 (21)	283 (21)	48 (6)
RF positivity, n (%)	374 (26)	359 (27)	68 (8)
anti-CarP positivity, n (%)	271 (19)	257 (19)	77 (9)

Of the total group of UA patients (n=1430), 760 patients have UA both according to the 1987 and 2010 criteria, 592 patients only have UA according to the 1987 criteria, and 78 patients only have UA according to the 2010 criteria. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CRP, C-reactive protein; IQR, interquartile range; RF, rheumatoid factor; SD, standard deviation; SJC, swollen joint count; symptom duration, time between symptom onset and inclusion in cohort; TJC, tender joint count; UA, undifferentiated arthritis.

The value of anti-CarP antibodies was first studied in the 1987-UA patients. The presence of anti-CarP antibodies at baseline was associated with progression to RA (OR 4.2, 95% CI 3.2–5.6), an association which remained when a correction was made for the presence of ACPA and RF (OR 1.7, 95% CI 1.2–2.4). There was no additional predictive value of anti-CarP antibody levels in anti-CarP-positive patients. The association of anti-CarP antibodies with progression to RA was then determined within the strata of patients with a similar ACPA and RF status. The majority of the UA patients (69%) were ACPA- and RF-negative; 7% (65/929) of these ACPA- and RF-negative patients had anti-CarP antibodies (Table 4.2). Within this subgroup, the presence of anti-CarP antibodies was statistically significantly associated with progression to RA (OR 2.1, 95% CI 1.3–3.7). When absolute risks were examined, the pre-test risk for RA development in the ACPA- and RF-negative subgroup was 21%, and this increased to 35% when anti-CarP antibodies were present (Table 4.2). When exploring the negative predictive value (NPV), the pre-test risk of not developing RA was 79% which was similar to the NPV of 80%.

Next, the predictive value of anti-CarP antibodies was studied within the 2010-UA patients. Here, anti-CarP antibodies at baseline were associated with progression to 2010-RA within 1 year (OR 2.9, 95% CI 1.4–5.8). However, when adjustment was made for the presence of ACPA and RF, there was no additive predictive value of

anti-CarP antibodies (OR 0.8, 95% CI 0.3–2.1). When analyzing groups of patients stratified according to the absence of ACPA and RF, the majority of 2010-UA patients were ACPA- and RF-negative (90%) and only 6% (49/755) of these patients had anti-CarP antibodies. Within this subgroup, no predictive value of anti-CarP antibodies was observed (Table 4.2). Evaluation of absolute risks in the ACPA- and RF-negative subgroup revealed that the pre-test risk of developing RA was 4% and the positive predictive value (PPV) was 2% when anti-CarP antibodies were present. Likewise, the pre-test risk of not developing RA in this subgroup was similar to the post-test risk (NPV) when patients tested negative for anti-CarP antibodies (both 96%, Table 4.2).

Table 4.2 Proportion of 2010-UA and 1987-UA patients progressing to RA within 1 year within groups of similar ACPA and RF status

1987-UA patients (n=1352)		1987-RA	no 1987-RA	%RA / %non-RA development (pre-test risks)†	OR (95% CI)	PPV (95% CI)	NPV (95% CI)
ACPA- RF- (n=929)	anti-CarP+	23	42	21/79	2.1	35	80
	anti-CarP-	176	688		(1.3-3.7)	(25-48)	(77-82)
ACPA+ RF- (n=64)	anti-CarP+	16	10	50/50	2.2	62	58
	anti-CarP-	16	22		(0.8-6.1)	(43-78)	(42-72)
ACPA- RF+ (n=140)	anti-CarP+	8	9	39/61	1.4	47	62
	anti-CarP-	47	76		(0.5-4.0)	(26-69)	(53-70)
ACPA+ RF+ (n=219)	anti-CarP+	107	42	71/29	1.2	72	31
	anti-CarP-	48	22		(0.6-2.2)	(64-78)	(22-43)
2010-UA patients (n=838)		2010-RA	no 2010-RA	%RA / %non-RA development	OR (95% CI)	PPV (95% CI)	NPV (95% CI)
ACPA- RF- (n=755)	anti-CarP+	1	48	4/96	0.5	2	96
	anti-CarP-	30	676		(0.1-3.5)	(0-11)	(94-97)
ACPA+ RF- (n=15)	anti-CarP+	1	2	13/87	5.5	33	92
	anti-CarP-	1	11		(0.2-129)	(6-79)	(65-99)
ACPA- RF+ (n=35)	anti-CarP+	0	4	17/83	Undefined	0	81
	anti-CarP-	6	25			(0-49)	(64-91)
ACPA+ RF+ (n=33)	anti-CarP+	9	12	42/58	1.1	43	58
	anti-CarP-	5	7		(0.2-4.4)	(24-63)	(32-81)

Patients were stratified according to the presence of different autoantibody combinations (ACPA-RF-, ACPA+RF-, ACPA-RF+, and ACPA+RF+); within these groups the predictive value of the presence of anti-carbamylated protein (anti-CarP) antibodies for progression to rheumatoid arthritis (RA) was determined, both within 2010-undifferentiated arthritis (UA) and 1987-UA patients. †Observed risk of RA development within ACPA and RF strata (pre-test risks), without information on anti-CarP status. ACPA, anti-citrullinated protein antibodies; CI, confidence interval; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; RF, rheumatoid factor.

Discussion

This study was performed to increase our understanding of the value of recently identified autoantibodies to predict RA development using accurate stratification for ACPA and RF. Anti-CarP antibodies were studied as an example. We observed that the presence of anti-CarP antibodies was statistically significantly associated with the development of RA within ACPA- and RF-negative 1987-UA patients. In this group, the risk of developing RA increased from 21% to 35% when anti-CarP antibodies were present. However, when RA was defined according to the 2010 criteria, anti-CarP antibodies were not associated with RA development and the presence of these autoantibodies did not increase the risk of RA development compared to the pre-test risks.

Although they used different study designs and entire early arthritis populations, two previous studies found 2.2% and 0.4% improved classification when adding anti-CarP antibodies to ACPA and RF, thus showing little additive benefit.[8,11] These findings are in line with our data.

Presumably this finding is explained by the fact that ACPA and RF are heavily weighted in the 2010 criteria. Consequently, the majority of UA patients are ACPA- and RF-negative and these patients can only fulfil the 2010 criteria if they develop >10 involved joints but they can fulfil the 1987 criteria over time with less extensive disease progression; hence the definition of the outcome matters. Additionally, autoantibodies frequently occur together (Figure 4.1), as has been shown for several novel autoantibodies.[3,5] These two explanations might also apply to other novel autoantibodies. Although novel autoantibodies other than anti-CarP antibodies were not evaluated here, we anticipate that similar findings will be obtained. Importantly, our findings relate to the earlier identification of patients with RA; novel autoantibodies can still be useful for other outcomes, such as radiographic progression.[7]

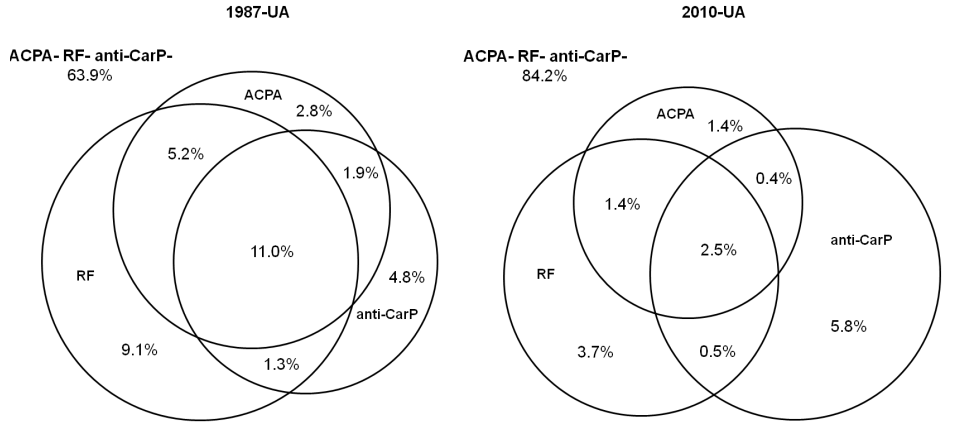

Figure 4.1 Concomitant presence of anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF), and anti-carbamylated protein (anti-CarP) antibodies in patients with 1987-undifferentiated arthritis (UA) and 2010-UA

Depicted are the percentages of the 1352 1987-UA (A) and the 838 2010-UA (B) patients positive for ACPA, RF, and/or anti-CarP antibodies.

Conclusion

More research is needed to identify early RA patients among (2010 criteria-negative) UA patients, but based on the composition of the current classification criteria it will be interesting to evaluate other, non-autoantibody biomarkers.

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5

Are MRI-detected erosions specific for RA? A large explorative cross-sectional study

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Abstract

Objectives

Magnetic resonance imaging (MRI) is recommended in the diagnostic process of rheumatoid arthritis (RA), to detect joint damage early. MRI-detected erosions are also present in symptom-free controls, especially at older age. It is unclear if RA-specific MRI-detected erosions can be distinguished from 'physiological' erosions in symptom-free individuals. This study compared MRI-detected erosions of RA patients with healthy controls and with other arthritides.

Methods

589 newly-presenting early arthritis patients (238 RA, 351 other arthritides) and 193 symptom-free controls underwent contrast-enhanced 1.5T MRI of unilateral metacarpophalangeal and metatarsophalangeal (MTP) joints. Total erosion score (according to RAMRIS), number, severity, location of erosions and simultaneous presence of MRI-detected inflammation (synovitis and/or bone marrow edema) were compared; participants were categorized in three age-groups (<40, 40-59, ≥60).

Results

RA patients had statistically significant higher total erosion scores than controls but scores of individual persons largely overlapped. Grade ≥2 erosions and MTP5-erosions were specific for RA (specificity 98-100% and 90-98% for different age-groups). MTP1-erosions were only specific if aged <40 (specificity 98%) and erosions with inflammation if aged <60 (specificity 91-100%). ≥1 of the mentioned erosions characteristics were present in 29% of RA patients. Comparing RA patients with other arthritides revealed that grade ≥2 erosions and MTP5-erosions remained specific for RA (specificity ≥89%) as well as MTP1-erosions if aged <40 (specificity 93%), in contrast to erosions combined with inflammation (specificity 49-85%).

Conclusions

Total erosion scores of individual persons were largely overlapping. Erosion characteristics specific for RA were identified, but were infrequently present. Caution is needed not to overestimate the value of MRI-erosions in the diagnostic process.

Introduction

Rheumatoid arthritis (RA) is characterized by joint inflammation that may lead to bone erosions. Traditionally erosions are evaluated using conventional radiographs. Recently it has been recommended by the EULAR imaging taskforce that magnetic resonance imaging (MRI) is valuable to detect erosions early.[1] Indeed MRI has shown to be more sensitive for structural damage in early RA than conventional radiographs.[2-9]

Radiographic erosions specific for RA are defined in the 2010 ACR/EULAR-criteria[10] as erosions seen in at least three separate joints at the proximal interphalangeal, the metacarpophalangeal (MCP), the wrist and metatarsophalangeal (MTP) joints (specificity >80%, sensitivity 15-29%).[11] However, for MRI-detected erosions a definition specific for RA has not yet been derived. Because MRI is more sensitive in detecting erosions than radiographic imaging, RA-specific MRI-detected erosions need to be characterized.

Previously it was shown that MRI-detected erosions are also observed in other rheumatic diseases and in healthy controls, especially at older age.[12-17] Thus, in order to prevent false-positive MRI-results, it is important to distinguish RA-specific erosions from other erosions.

This cross-sectional study compared erosions in MCP and MTP joints as detected on MRI (evaluated using the rheumatoid arthritis MRI scoring system (RAMRIS)) between early RA patients at the time of diagnosis and symptom-free controls for different characteristics: besides the total erosion score, also the number, severity and location of erosions and the simultaneous presence of MRI-detected inflammation (synovitis and/or bone marrow edema (BME)) were compared. Second, RA patients were also cross-sectionally compared to early arthritis patients that presented with other diagnoses. Within patients that presented with undifferentiated arthritis (UA), erosions were compared between patients that did and did not progress to RA during the first year. All analyses were done with the ultimate aim to identify features of MRI-detected erosions that are specific for RA.

Methods

Patients

598 patients who presented with early arthritis and were included in the Leiden Early Arthritis Clinic (EAC) between 2010 and 2014 were studied. The EAC is an inception cohort including patients with clinically confirmed arthritis and symptom duration <2 years. At baseline questionnaires were administered, joint counts and blood samples were collected and MRI was performed.[18] Nine patients were excluded because no contrast agent was administered. Two weeks after inclusion, when results of regular investigations were known (this did not include information on MRI-results), the initial diagnosis of the patients was documented by the rheumatologists. The clinical diagnosis of RA was verified by fulfilling the 1987 or 2010 criteria at baseline.[10,19] Of the 589 patients 238 patients had RA. The diagnoses of the remaining group with other arthritides (n=351) were UA (n=192), reactive arthritis (n=22), (pseudo)gout (n=15), psoriatic arthritis (n=34), inflammatory osteoarthritis (OA) (n=35), Lyme arthritis (n=3), paramalignant arthritis (n=1), SLE (n=4), other systemic disorder (n=7), MCTD, vasculitis (n=2), sarcoidosis (n=3), spondylarthropathy with peripheral arthritis (n=5), RS3PE (n=10), and other diagnosis (n=18).

In addition, 193 symptom-free controls were recruited by advertisements in local newspapers and websites as previously reported.[13] They had no history of RA or other inflammatory rheumatic diseases, no joint symptoms during the last month, no recent trauma (<1 year prior to MRI) and no arthritis at physical examination.

Both studies were approved by the local medical ethics committee. All patients and controls signed informed consent.

MR imaging and scoring

At baseline MRI of the 2nd-5th MCP and 1st-5th MTP joints on the most painful side or in case of symmetric symptoms and in healthy controls on the dominant side was performed. MR imaging was performed on a MSK Extreme 1.5T extremity MRI system (General Electric, Wisconsin, USA). The MRIs of all subjects were made on the same scanner. Coronal T1-weighted fast spin-echo (FSE) and contrast-enhanced coronal and axial T1-weighted FSE with frequency-selective fat suppression were obtained. Further details on the scan protocol are provided in the online Supplementary methods. Erosions, BME and synovitis were scored according to the RAMRIS method, with the exception that BME was assessed on a contrast-

enhanced T1-weighted fat-suppressed sequence.[20] According to the RAMRIS method erosions were defined as sharply marginated bone lesions, with correct juxta-articular localization and typical signal characteristics, which are visible in two planes with a cortical break seen in at least one plane. All bones were scored separately for erosions on a scale 0-10, based on the proportion of eroded bone (0: no erosion, 1: 1-10% of bone eroded, 2: 11-20%, etc.). The total erosion score was calculated by summing the erosion score in the MCP and MTP joints (range 0-180). Each MRI was scored by two readers, blinded to any clinical data. Intra-reader intraclass correlation coefficients (ICCs) and interreader ICCs were ≥0.86 (see online Supplementary methods).

Erosion characteristics

The total erosion score (hence a combination of number of erosions and severity), number, severity and location of erosions were studied on the person level. The presence of concomitant inflammation was studied. This comprised the presence of BME in the same bone or the presence of synovitis around the same bone as where the erosion was located. These analyses were done on person and on bone level. For the total erosion score the mean of two readers was used. When assessing number, severity, location and the combination of erosions with inflammation, MRI-erosions were considered present when the mean of both readers was ≥1 at a specific bone. Grade ≥2 erosions indicate that >10% of the bone is eroded.

Statistical analyses

First, total erosion scores of RA patients were compared with scores of controls. A linear regression analysis adjusted for age and gender was used with the total erosion score as outcome and group (RA/healthy control) as independent variable. Erosion scores were logtransformed (log10(score+1)) to approximate a normal distribution. The reported effect sizes were back-transformed to the normal score and indicated how many times the erosion scores of RA patients were higher than that of controls. Thereafter, patients were stratified in three age groups (<40, 40-59, ≥60 years) and frequencies of erosion characteristics were compared between groups. Test characteristics were determined. Similar analyses were performed comparing RA patients with other arthritides. Finally the diagnostic value of MRI-detected erosions in UA patients was assessed. SPSS version 23.0 (IBM) was used. P-values <0.05 were considered significant.

Results

Patient characteristics

Baseline characteristics of patients and symptom-free controls are presented in Table 5.1.

Table 5.1 Baseline characteristics of RA patients, symptom-free controls and patients with other arthritides

	RA patients (n=238)	Symptom-free controls (n=193)	Patients with other arthritides (n=351)
Age in years, mean (SD)	56 (15)	50 (16)	54 (16)
Female, n(%)	159 (67)	136 (70)	204 (58)
Symptom duration in weeks, median (IQR)	15 (8-29)	NA	9 (4-26)
66-SJC, median (IQR)	6 (2-11)	NA	2 (1-4)
68-TJC, median (IQR)	9 (5-15)	NA	3 (2-8)
CRP (mg/ml), median (IQR)	9 (3-21)	NA	4 (3-13)
RF positivity, n(%)	147 (64)	NA	39 (12)
ACPA positivity, n(%)	123 (52)	NA	14 (4)

ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; IQR, interquartile range; NA, not applicable; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; SJC, 66-swollen joint count; TJC, 68-tender joint count.
Some serology data were missing as follows: in RA patients RF n=10, ACPA n=1; in patients with other arthritides: RF n=15, ACPA n=12.

At group level RA patients have slightly higher MRI-detected erosion scores than symptom-free controls but on the individual level there is large overlap

First the total erosion scores were evaluated. In both the group of RA patients and that of symptom-free controls the MRI-erosion score was associated with age (Figure 5.1A, Supplementary table 5.1). When comparing the erosion scores of RA patients and controls, RA patients had 1.20 (95% CI 1.08-1.33, $p<0.001$) times higher erosion scores than controls, independent of age and gender. This effect size indicates that RA patients had in general a 20% higher total erosion score than controls. Despite the significant difference there was large overlap of MRI-erosion scores between RA patients and controls, as visually no separate clustering of groups was observed (Figure 5.1A). Thus total erosion scores could not differentiate RA patients from controls on the individual level.

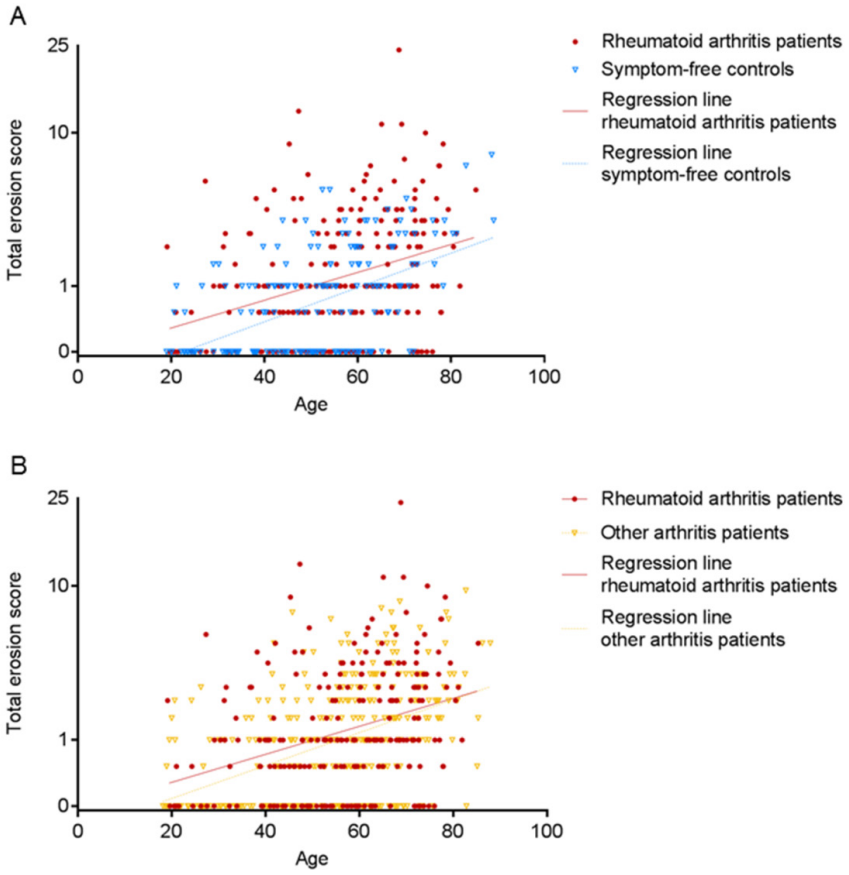


Figure 5.1 MRI-detected erosions in MCP and MTP joints in relation to age in RA patients and in controls (A) and in patients with other arthritides (B); both figures show overlap at the individual level

Linear regression analyses were performed with the mean total erosion score as detected by magnetic resonance imaging as outcome and group (RA patients and healthy controls (A) or other arthritides (B)), age and gender as independent variables. Y-axis is log-transformed. MCP; metacarpophalangeal; MTP; metatarsophalangeal.

Grade ≥2 MRI-erosions are more prevalent in RA patients than in symptom-free controls

Then other erosion characteristics were studied to search for RA-specific characteristics. Because of the association with age, all analyses were stratified for age group (<40, 40-59 and ≥60 years). Since the total erosion score is a combination of the number of erosions and severity, both characteristics were evaluated separately. The median total number of erosions was 1.0 (IQR 0-2.0) for

RA patients and 0 (IQR 0-1.0) for symptom-free controls (Mann-Whitney U-test: p=0.001). Within the different age groups there were no significant differences in the two oldest groups. In the group <40 years, RA patients had more erosions than controls (median 0 (IQR 0-1.0) versus 0 (IQR 0-0), p=0.007) though differences were too small to identify a number of MRI-detected erosions that was specific for RA. To determine whether grade ≥2 erosions were RA-specific, the frequency of grade ≥2 erosions was considered per joint location (Supplementary table 5.2). This revealed that grade ≥2 erosions were almost exclusively present in RA (specificity 98-100% for different age groups, Table 5.2). However within all age groups they were only sporadically observed in RA at disease presentation (sensitivity 5-10%). Evaluation on person level showed that 8% of the RA patients had at least one grade ≥2 erosion in an MCP and/or MTP joint, while in controls this was only 1% (Table 5.3). Thus the presence of grade ≥2 erosions was highly specific for RA, but also infrequent in RA at disease presentation.

Table 5.2 Test characteristics of grade ≥2 erosions (a), an erosion in MTP5 (b), an erosion in MTP1 (c) and erosions in combination with inflammation (d) for RA

		RA patients	RA patients vs. healthy controls	RA patients vs. patients with other arthritides
		sensitivity (95% CI)	specificity (95% CI)	specificity (95% CI)
a	**Test characteristics grade ≥2 erosion**			
	<40 years	9% (3-24)	100% (93-100)	100% (95-100)
	40-59 years	5% (2-12)	99% (94-100)	96% (91-98)
	≥60 years	10% (6-17)	98% (90-100)	96% (91-98)
b	**Test characteristics erosion in MTP5**			
	<40 years	24% (13-41)	98% (90-100)	100% (95-100)
	40-59 years	9% (5-17)	90% (82-95)	89% (83-93)
	≥60 years	16% (10-24)	92% (82-97)	90% (84-94)
c	**Test characteristics erosion in MTP1**			
	<40 years	18% (9-34)	98% (90-100)	93% (84-97)
	40-59 years	19% (12-28)	86% (77-91)	77% (69-83)
	≥60 years	36% (27-45)	63% (50-75)	66% (57-73)
d	**Test characteristics of erosion in combination with inflammation**			
	<40 years	33% (20-50)	100% (93-100)	85% (75-92)
	40-59 years	24% (17-33)	91% (83-95)	69% (61-76)
	≥60 years	56% (47-65)	71% (58-82)	49% (41-57)

RA, rheumatoid arthritis; MTP, metatarsophalangeal joint; CI, confidence interval.

Table 5.3 Frequencies of RA patients and controls with grade ≥2 erosions and with erosions with the simultaneous presence of local inflammation in an MCP and/or MTP joint; analyses on person level

				Grade ≥2 erosions		Erosions with inflammation	
			Nr. of persons with erosions	No grade ≥2 erosions	grade ≥2 erosions	Erosion+ inflammation-	Erosion+ inflammation+
RA	<40 years	(n=33)	14	11 (79%)	3 (21%)	3 (21%)	11 (79%)
	40-59 years	(n=96)	39	34 (87%)	5 (13%)	16 (41%)	23 (59%)
	≥60 years	(n=109)	79	68 (86%)	11 (14%)	18 (23%)	61 (77%)
		238					
Control	<40 years	(n=51)	9	9 (100%)	0 (0%)	9 (100%)	0 (0%)
	40-59 years	(n=90)	36	35 (97%)	1 (3%)	28 (78%)	8 (22%)
	≥60 years	(n=52)	36	35 (97%)	1 (3%)	21 (58%)	15 (42%)
		193					

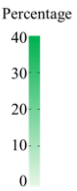
The presence of grade ≥2 erosions and erosions with inflammation (BME and/or synovitis) was evaluated per MCP and MTP bone according to the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring system. Grade ≥2 erosions indicate that >10% of the bone is eroded. The presence of BME and/or synovitis was defined as a score of ≥1. BME, bone marrow edema; MCP, metacarpophalangeal; MTP, metatarsophalangeal; RA, rheumatoid arthritis.

MTP5 and MTP1 are more often affected in RA patients than in symptom-free controls

Then the location (the affected MCP or MTP joint) was assessed (Table 5.4). Both in RA patients and controls most erosions were located in the proximal part of the MCP and MTP joints: in RA patients 82-95% of the erosions was located proximal in the joint and in controls this was 81-100% for the different age groups. As presented in Table 5.4, overall the MCP and MTP bones that were frequently affected in RA patients were also frequently affected in healthy controls. For instance MCP2 and MCP3 were predilection sites for MRI-detected erosions in RA, but also in controls. However there were also some differences: erosions in MTP5 were more frequently present in RA patients than in controls in most age groups (specificity 90-98% for different age groups, Table 5.2). In addition, erosions in MTP1 in the age group <40 almost exclusively occurred in RA (specificity 98%); the specificity was lower in older age groups (specificity 86% if aged 40-59 and 63% if aged ≥60). Examples of MRI-detected erosions are shown in Figure 5.2.

Table 5.4 Location of erosions in bones of the MCP and MTP joints of RA patients and symptom free controls, depicted per age category (18-39, 40-59, ≥60 years)

Erosions		<40 years		40-59 years		≥60 years	
		RA	Control	RA	Control	RA	Control
		n=33	n=51	n=96	n=90	n=109	n=52
MCP 2	proximal	6	6	7	11	23	23
	distal	0	0	4	2	9	10
MCP 3	proximal	9	8	15	12	30	23
	distal	0	0	0	1	5	4
MCP 4	proximal	3	0	3	2	9	8
	distal	0	0	0	0	0	0
MCP 5	proximal	0	2	5	6	20	13
	distal	0	0	0	0	1	6
MTP 1	proximal	18	2	19	14	36	37
	distal	0	0	2	0	11	6
MTP 2	proximal	0	0	2	0	4	0
	distal	3	0	0	1	4	0
MTP 3	proximal	0	0	2	0	6	0
	distal	0	0	0	0	2	0
MTP 4	proximal	3	0	3	0	1	0
	distal	0	0	0	0	0	0
MTP 5	proximal	24	2	9	10	16	8
	distal	0	0	2	0	1	2



Values are the percentages of persons with an erosion of all persons in that age category.
The presence of an erosion is defined as an erosion score of at least 1 in that bone.
MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

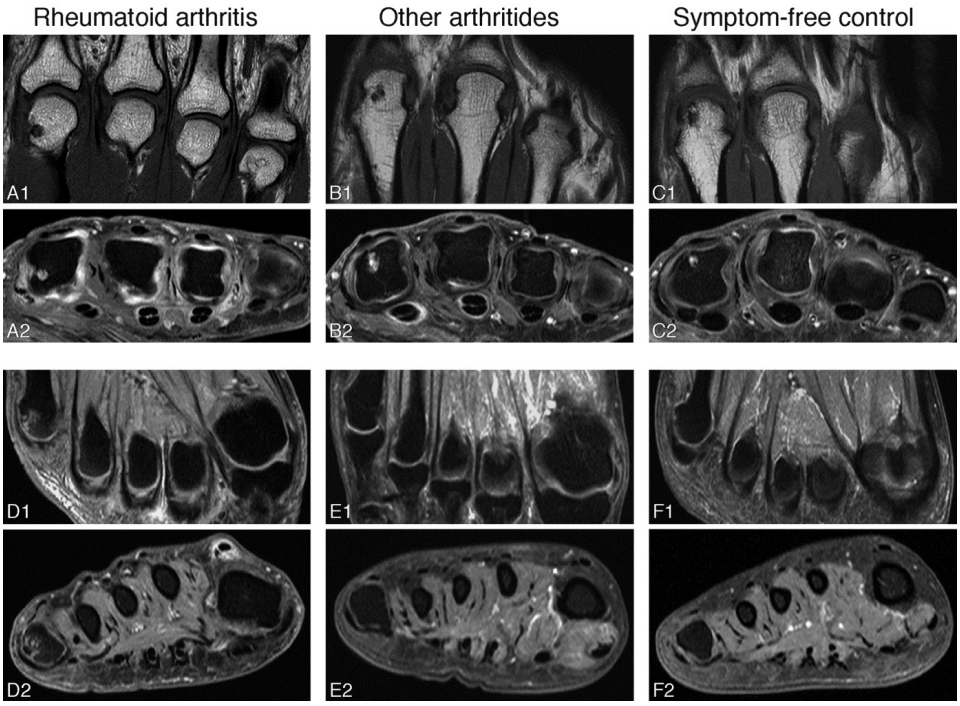


Figure 5.2 Examples of erosions in RA patients, patients with other arthritides and symptom-free controls

MR images of RA patients (A, D), patients with other arthritides (B, E) and symptom-free controls (C, F). Examples of erosion in MCP2 (A-C) and MTP5 are shown (D). Erosions in MCP2 were observed in all different groups (A-C), while erosions in MTP5 were mainly observed in RA patients (D). The erosion shown in MTP5 (D) is accompanied by the presence of bone marrow edema. Patient B was diagnosed with gout. Person C was aged 48 years. Coronal (A1, B1, C1, D1, E1, F1) and axial (A2, B2, C2, D2, E2, F2) images are shown. MRI sequences included coronal T1-weighted fast spin-echo (FSE) sequences and axial T1-weighted FSE sequences with fat suppression after contrast enhancement. MCP, metacarpophalangeal joint; MRI, magnetic resonance imaging; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

Erosions with the simultaneous presence of BME and/or synovitis are more frequent in RA patients than in symptom-free controls

Then we questioned whether the combined presence of erosions with surrounding inflammation was specific for RA. At bone level, in RA patients 33% (95/285) of the total number of MCP and MTP bones with erosions only had erosions without synovitis and/or BME while in controls this was 77% (105/136, Table 5.5). Similarly, when analysed on person level, 16% of the RA patients only had erosions without inflammation and 40% had at least one erosion with inflammation in that same joint while in controls this was 30% and 12%, respectively (Table 5.3). When

analysing the different age groups it appeared that within the age group <40 years, the simultaneous presence of erosions with inflammation was exclusively observed in RA patients (specificity 100%). In the age group 40-59 years the specificity was 91% and it was lower in persons aged ≥60 (specificity 71%) since in this age group erosions with inflammation were also observed in healthy controls (Table 5.2). Thus the presence of erosions with inflammation was specific for RA, but only if aged <60.

Altogether, the presence of grade ≥2 erosions and MTP5-erosions were specific for RA in all age groups, erosions with inflammation were specific for RA if aged <60 and MTP1-erosions if aged <40. Although these erosion characteristics were highly specific for RA only 29% of all RA patients had ≥1 erosion(s) with ≥1 of these characteristics.

Table 5.5 Frequencies of erosions in combination with inflammation in MCP and MTP bones of symptom free controls and RA patients; analysis on bone level

		Total nr. of MCP and MTP bones evaluated	Nr. of MCP and MTP bones with erosions				Nr. of MCP and MTP bones without erosions
			Erosion+ BME- Synovitis-	Erosion+ BME+ Synovitis-	Erosion+ BME- Synovitis+	Erosion+ BME+ Synovitis+	
RA	<40 years	594	7 (32%)	7 (32%)	4 (18%)	4 (18%)	572
	40-59 years	1728	28 (39%)	10 (14%)	12 (17%)	21 (30%)	1657
	≥60 years	1962	60 (31%)	12 (6%)	65 (34%)	55 (29%)	1770
		4284					3999
Control	<40 years	918	10 (100%)	0 (0%)	0 (0%)	0 (0%)	908
	40-59 years	1620	45 (83%)	2 (4%)	5 (9%)	2 (4%)	1566
	≥60 years	936	50 (69%)	7 (10%)	9 (13%)	6 (8%)	864
		3474					3338

Values are the number of MCP and MTP bones with erosions and without erosions. MCP and MTP bones with erosions are divided in subgroups of erosions without BME and synovitis and with BME and/or synovitis. Erosions, BME and synovitis were defined as a score of ≥1 according to the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring system. BME, bone marrow edema; MCP, metacarpophalangeal; MTP, metatarsophalangeal; RA, rheumatoid arthritis.

Erosions in MTP5 and grade ≥2 erosions in all age groups and erosions in MTP1 if aged <40 remain specific for RA when compared to patients with other arthritides

Thus far, different erosion characteristics were compared between RA patients and controls revealing some RA-specific characteristics. The next question is whether these characteristics are truly RA-specific or are also present in other arthritides. Therefore, all analyses were repeated with patients with other arthritides as reference group. The total erosion scores of both patient groups were not significantly different (beta 0.92 95% CI 0.84-1.01, Figure 5.1B). Comparison of the different erosion characteristics showed that the presence of grade ≥2 erosions was RA-specific in all age groups (specificity 100% if aged <40 and 96% if aged 40-59 and ≥60, Table 5.2, Supplementary table 5.3). Also MTP5-erosions were highly specific for RA in all age groups (specificity 100% if aged <40, 89% if aged 40-59 and 90% if aged ≥60, Table 5.2, Supplementary table 5.4). The specificity of MTP1-erosions was 93% in patients aged <40 but at higher age specificity decreased to 66%. Erosions with inflammation were less specific for RA (specificity 49-85% within different age groups) as these were also present in other arthritides. Thus, erosions with inflammation were not RA-specific, but MTP5-erosions and grade ≥2 erosions were specific in all age groups and MTP1-erosions in patients aged <40. 21% of RA patients had ≥1 erosion(s) with these characteristics (sensitivity 21%). Additionally, of all patients with erosions with one of these three finally identified features, 53% fulfilled criteria for RA (PPV 53%) and of all patients without such erosions, criteria were not fulfilled in 62% (NPV 62%).

MRI-detected erosions do not contribute to the identification of UA patients that will progress to RA

Finally, the value of MRI-detected erosions was evaluated within UA patients. Of the UA patients, 15% (28/192) fulfilled criteria for RA after one year. Of these patients 11% had an RA-specific erosion at baseline, whereas 9% of the non-convertors had an RA-specific erosion (OR 1.3 95% CI 0.3-4.8).

Discussion

Radiographic erosions specific for RA have been defined as the presence of ≥3 radiographic erosions on MCP, PIP, wrist or MTP joints and their presence is considered sufficiently specific to classify RA.[11] MRI is a sensitive imaging modality that depicts cortical defects and therefore is suitable to detect erosive damage.

Thus far it was unknown which MRI-detected erosions on hand and foot joints are specific for RA. This large cross-sectional MRI-study showed that on the group level, RA patients had higher MRI-detected erosion scores in MCP and MTP joints than controls, but also that there was large overlap on the individual level. Several erosion characteristics were studied in detail; this was done within three age strata as the total MRI-erosion score was associated with age. Compared to controls from the general population, four characteristics were identified as RA-specific: grade ≥2 erosions, MTP5-erosions, MTP1-erosions if aged <40 and erosions with local inflammation if aged <60. At least one of these characteristics is present in 29% of RA patients.

Subsequently RA patients were compared to early arthritis patients with other diagnoses, because studies comparing established cases and healthy controls will reveal the maximal contrast. Differences are often smaller when more clinically relevant patient groups are studied.[21,22] Indeed we observed that some erosion characteristics that were specific for RA when compared to controls were not specific when RA was compared with other arthritides. This was most prominent for the combined presence of erosions with inflammation. Nonetheless, some characteristics (grade ≥2 erosions, MTP5-erosions, MTP1-erosions in persons aged <40) were RA-specific in both settings. 21% of RA patients had ≥1 erosion(s) with ≥1 of these characteristics.

Although some erosion characteristics were identified as RA-specific, an important overlap between early RA patients and controls was observed. It has been recommended that novel imaging modalities, such as MRI, can be used to detect erosions early.[1] The present data show that if all MRI-detected erosions (according to RAMRIS) would be considered as characteristic for RA or disease, this would yield many false-positive results.

We used the RAMRIS-definition of erosions that basically evaluated the volume of the erosion in relation to the assessed bone. Others showed that small lesions on high-resolution peripheral quantitative computed tomography were not entirely specific for RA and suggested that lesions >1.9 mm in diameter were highly specific.[23,24] It remains to be determined if a phenotypic definition of MRI-erosions, for instance one that includes a description of the size of the cortical break, will be more discriminative; this is subject of further studies and is also considered within an ongoing EULAR taskforce.

Some of the findings on MRI-detected erosions are in line with previous findings on radiographic erosions. Radiographic erosions have been shown to occur more frequently at disease onset with higher age.[25-31] MTP5 has been shown as a predilection site for RA-related erosions as well.[32-34] We observed that the large majority of erosions (both in RA and in the other groups studied) were located in the proximal bone of the joint which is completely in line with previous findings.[23,33,35]

Erosive lesions in symptom-free controls have also been reported in other studies.[12] The nature of these lesions is unclear. Because of the association with age, degenerative subchondral bone cysts may be one of the explanations. In addition a very recent study, evaluating bone microstructure of MCP joints using high-resolution tomography and microCT, demonstrated that the number of so called cortical microchannels (linking the synovial and bone marrow compartments) was higher in RA patients than in healthy controls and was associated with erosions and with age.[36] It is intriguing to speculate that these channels have a causal role in erosion development, both in RA and controls. Another possibility is that mechanical strains are involved in erosion development, since erosions were frequently located in the foot (49% of the erosions in RA patients and 38% in symptom-free controls). However, a pathophysiological explanation for the findings done in symptom-free persons is beyond the scope of this study.

The location of erosions within the bone was not studied here. This information could not be discerned using RAMRIS as this method evaluates the volume of the erosive lesion per bone. However, previous studies have shown that the majority of erosive MRI-lesions in MCP joints occurred adjacent to the radial collateral ligaments, both in RA patients and in healthy controls.[37,38] Similar observations were done in a study in RA patients and healthy controls on the location of erosions as detected on CT.[23] The location of erosions in the symptom-free controls that were studied here has been reported previously,[13] and showed that also in these persons erosions were present adjacent to the collateral ligaments and were not situated centrally in the bone. Because of these previous reports, showing no difference in location of erosions within the bone between RA patients and controls, we anticipated that this characteristic will not result in further discrimination of RA-specific erosions from other erosions.

Cross-sectional analyses revealed that of all patients with an erosion that was identified as characteristic for RA 53% actually had RA (PPV). Likewise, 62% of all patients without such erosions did not have RA (NPV), whereas 38% did fulfil

criteria for RA. These data illustrate that the absence or presence of RA-specific erosions at disease presentation are of moderate value to identify patients that fulfil criteria for RA at the same point in time.

Longitudinal analysis within UA patients suggested that the presence of RA-specific erosions was also not predictive for the development of RA. However, this analysis was of limited power. Additionally, other outcomes, such as the start of disease-modifying antirheumatic drugs (DMARDs), should be studied, since DMARD treatment might hamper progression to RA. Finally, it was not possible to study the different RA-specific erosion features separately due to the limited number of patients. Further studies are warranted.

We studied an early RA-population. 36% of the patients was RF-negative and 48% were ACPA-negative which is comparable to other early RA cohorts.[39,40] Our population is somewhat different from RA patients included in clinical trials where generally a selection of RA patients is included.

A limitation of this study is that it was cross-sectional in nature and that imaging follow-up was not studied. Sensitivity of readers could have been a problem and could be equally present in the three groups. The presence of serial MRI-data facilitates the differentiation of erosions from vascular channels and entheseal attachments, as these should not change during follow-up. Erosions in contrast could progress over time, although this progression may also have been hampered by up-to-date treatment strategies. Serial MRIs were not made but would have been beneficial to evaluate if some erosions were falsely identified as such. However if MRI will be used for early identification of patients in clinical practice, single MRI-measurements will be made.

In conclusion, MRI-detected erosions (according to the RAMRIS definition) in MCP and MTP joints are not confined to RA, but also present in other arthritides and in symptom-free persons from the general population. On the individual level there was a large overlap. Some erosion characteristics were identified as specific for RA (grade ≥2 erosions, MTP5-erosions, and MTP1-erosions if aged <40), though these occurred in a minority (21%) of the patients. Longitudinal MR-imaging may improve specificity; however this was not tested in this study. The present data imply that if single measurements with novel imaging modalities such as MRI are used for the early detection of structural damage in clinical practice, the risk of false-positive findings should be considered.

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Supplementary material

Supplementary methods are available at the website of Annals of the Rheumatic Diseases.

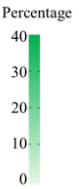
Supplementary table 5.1 Median and mean values of the total erosion score in MCP and MTP joints

	RA	Symptom-free control	Other arthritides
Median (IQR)	1.0 (0.4-2.5)	0.5 (0-1.5)	1.0 (0-2.0)
<40 years	0.5 (0-1.3)	0 (0-0.5)	0 (0-0.9)
40-59 years	0.5 (0-1.4)	0.5 (0-1.0)	1 (0-2.0)
≥60 years	2.0 (1.0-3.5)	1.5 (0.5-2.5)	1.5 (0.5-3.0)
Mean (SD)	1.8 (2.4)	0.9 (1.2)	1.3 (1.5)
<40 years	1.0 (1.2)	0.3 (0.5)	0.4 (0.6)
40-59 years	1.2 (1.8)	0.8 (1.0)	1.2 (1.3)
≥60 years	2.5 (2.9)	1.7 (1.5)	1.9 (1.8)

Presented are the median and mean total erosion scores in MCP en MTP joints for RA patients, symptom-free controls and patients with other arthritides, within different age groups. IQR, interquartile range; MCP, metacarpophalangeal; MTP, metatarsophalangeal; RA, rheumatoid arthritis; SD, standard deviation.

Supplementary table 5.2 Frequencies of erosions with score ≥2 in the MCP and MTP joints of RA patients and symptom free controls, depicted per age category (18-39, 40-59, ≥60 years)

Erosions		<40 years		40-59 years		≥60 years	
		RA	Control	RA	Control	RA	Control
		n=33	n=51	n=96	n=90	n=109	n=52
MCP 2	proximal	0	0	0	0	3	0
	distal	0	0	0	0	0	0
MCP 3	proximal	3	0	2	1	3	2
	distal	0	0	0	0	0	0
MCP 4	proximal	0	0	0	0	0	0
	distal	0	0	0	0	0	0
MCP 5	proximal	0	0	0	0	0	0
	distal	0	0	0	0	0	0
MTP 1	proximal	0	0	0	0	3	0
	distal	0	0	0	0	1	0
MTP 2	proximal	0	0	1	0	0	0
	distal	0	0	0	0	1	0
MTP 3	proximal	0	0	2	0	1	0
	distal	0	0	0	0	0	0
MTP 4	proximal	0	0	0	0	0	0
	distal	0	0	0	0	0	0
MTP 5	proximal	6	0	1	0	3	0
	distal	0	0	1	0	1	0



Values are the percentages of persons with an erosion score of at least 2 of all persons in that age category. MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

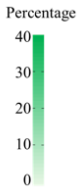
Supplementary table 5.3 Frequencies of RA patients and patients with other arthritides with grade ≥2 erosions and with erosions with the simultaneous presence of local inflammation in an MCP and/or MTP joint; analyses on person level

				Grade ≥2 erosions		Erosions with inflammation	
			Nr. of persons with erosions	No grade ≥2 erosions	grade ≥2 erosions	Erosion+ inflammation-	Erosion+ inflammation+
RA	<40 years	(n=33)	14	11 (79%)	3 (21%)	3 (21%)	11 (79%)
	40-59 years	(n=96)	39	34 (87%)	5 (13%)	16 (41%)	23 (59%)
	≥60 years	(n=109)	79	68 (86%)	11 (14%)	18 (23%)	61 (56%)
		238					
Other arthritides	<40 years	(n=68)	17	17 (100%)	0 (0%)	7 (41%)	10 (59%)
	40-59 years	(n=146)	74	68 (92%)	6 (8%)	29 (39%)	45 (61%)
	≥60 years	(n=137)	99	93 (94%)	6 (6%)	29 (29%)	70 (71%)
		351					

The presence of grade ≥2 erosions and erosions with inflammation (BME and/or synovitis) was evaluated per MCP and MTP bone according to the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring system. Grade ≥2 indicate that >10% of the bone is eroded. The presence of BME and/or synovitis was defined as a score of ≥1. BME, bone marrow edema; MCP, metacarpophalangeal; MTP, metatarsophalangeal; RA, rheumatoid arthritis.

Supplementary table 5.4 Location of erosions in bones of the MCP and MTP joints of RA patients and patients with other arthritides, depicted per age category (18-39, 40-59, ≥60 years)

Erosions		<40 years		40-59 years		≥60 years	
		RA	other arthritides	RA	other arthritides	RA	other arthritides
		n=38	n=63	n=112	n=130	n=134	n=112
MCP 2	proximal	5	2	10	6	22	26
	distal	0	0	4	2	9	8
MCP 3	proximal	8	8	16	22	31	26
	distal	0	2	0	0	4	5
MCP 4	proximal	5	0	3	4	8	6
	distal	3	0	0	0	0	4
MCP 5	proximal	0	2	5	5	18	14
	distal	0	2	1	0	2	1
MTP 1	proximal	16	8	21	22	36	34
	distal	0	6	2	2	10	9
MTP 2	proximal	0	2	2	2	3	3
	distal	3	0	0	1	4	1
MTP 3	proximal	0	0	2	1	4	1
	distal	0	0	0	2	1	1
MTP 4	proximal	3	0	3	2	1	0
	distal	0	0	0	0	0	1
MTP 5	proximal	21	0	11	10	15	10
	distal	0	0	2	0	1	0



Values are the percentages of patients with an erosion of all patients in that age category. The presence of an erosion is defined as an erosion score of at least 1 in that bone. MCP, metacarpophalangeal joint; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis.

6

Evaluation of the predictive accuracy of MRI-detected erosions in hand and foot joints in patients with undifferentiated arthritis

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Ann Rheum Dis. 2019;78(1):144-146.

Radiographic erosions are a clear hallmark of rheumatoid arthritis (RA). The European League Against Rheumatism (EULAR) definition of radiographic erosive disease has a high specificity, and its fulfilment alone is sufficient to classify RA.[1] However, the sensitivity of radiography to detect erosions early in the disease is low. Other imaging techniques, such as magnetic resonance imaging (MRI), are more sensitive to detect erosions than radiography and are therefore recommended by a EULAR imaging task force.[2] To determine the specificity of MRI-detected erosions, we recently compared erosions in the metacarpophalangeal (MCP) and metatarsophalangeal (MTP) joints (scored according to the RA MRI Scoring System (RAMRIS)[3]) of patients presenting with RA with those of symptom-free persons and patients presenting with arthritides other than RA.[4] MRI-detected erosions were present in all groups; therefore, the specificity of the presence of any MRI-detected erosions was low. By evaluating different erosion features, a few features were identified as specific for RA; these were severe erosions (grade ≥2, defined as >10% of bone eroded), erosions in MTP5 and erosions in MTP1 in persons aged <40. A subsequent and clinically relevant question is whether MRI-detected erosions in patients presenting with undifferentiated arthritis (UA) are valuable in predicting future progression to RA. This was explored to a limited extent in our previous study but as the number of patients with UA was limited (n=192), the predictive value of the different 'RA-specific erosions' could not be studied. In addition, the outcome was fulfilment of classification criteria but start of disease-modifying antirheumatic drugs (DMARDs) was not considered, while DMARD treatment might have hampered progression to fulfilment of RA classification criteria. Finally, MRI-detected erosions were only evaluated in the MCP and MTP joints and not in wrist joints, while erosions in the wrist are prevalent. To evaluate the predictive accuracy of MRI-detected erosions more thoroughly, we continued with a larger longitudinal study. In order to also include erosions in the wrist, we also performed a cross-sectional comparison between patients with early RA and patients with other arthritides to search for erosion features of wrist joints which are RA specific.

All studied patients were consecutively included in the Leiden Early Arthritis Clinic cohort. Inclusion required the presence of clinically confirmed inflammatory arthritis and symptom duration less than 2 years.[5] At baseline 1.5T MRI of the 2nd-5th MCP, wrist and 1st-5th MTP joints was performed as described.[4] Erosions were scored on a scale 0–10 according to the RAMRIS system.[3] Wrist erosions specific for RA were assessed by performing cross-sectional comparisons of MRI-detected erosions in the wrist in 238 patients with RA and 351 patients with other arthritides

who were included between 2010 and 2014; the number, location and severity of erosions as well as concomitant bone marrow edema (BME) were evaluated. Thereafter, the predictive value of MRI-detected erosions in MCP, wrist and MTP joints in 286 patients with UA (using the 2010 criteria to classify RA), included between 2010 and 2016, was evaluated. The predictive accuracy of the presence of any MRI-detected erosions, defined as score ≥1 by both readers, as well as of the presence of RA-specific erosions (as defined previously for MCP and MTP joints or as studied here for the wrist) was assessed.[4]

Ninety-four per cent of the 286 2010-UA patients were anti-citrullinated protein antibody (ACPA)-negative (Supplementary table 6.1), which is in line with other descriptions of the population of 2010-UA patients.[6,7] Patients were followed for 1 year on RA development, defined as fulfilling the 2010 criteria or the start of DMARDs because of a clinical diagnosis of RA. The latter was added as ACPA-negative patients need >10 involved joints to fulfil the 2010 criteria which could be hampered by DMARD treatment. One hundred and twenty-eight (45%) patients with UA developed the outcome, of which 111 had a clinical diagnosis of RA and started DMARDs and 17 fulfilled the 2010 criteria.

First, we searched for MRI-detected wrist erosions that were specific for RA. The median total number of erosions in the wrist was 1.0 (IQR 0–3.0) for patients with RA and 1.0 (IQR 0–2.0) for patients with other arthritides (Mann-Whitney U test: p=0.82). Severe erosions, defined as grade ≥2, were infrequent and present at a similar rate in patients with RA and patients with other arthritides (5% and 6%, respectively; Supplementary table 6.2). With respect to the location, erosions were most frequently observed in the capitate, triquetrum, lunate and scaphoid, especially at increasing age of onset; however, the frequency was not different in patients with RA and patients with other arthritides (Supplementary table 6.3). Finally, the combined presence of erosions with BME within the same bone was evaluated. This combination was more prevalent with increasing age of onset, but frequencies were comparable in both groups (30% of both patients with RA and patients with other arthritides, Supplementary table 6.2). Altogether, no RA-specific features of MRI-detected erosions located in the wrist could be identified.

Next, the predictive value of MRI-detected erosions was evaluated in patients with UA. Any MRI-detected MCP and MTP erosions were present in 49% of the 286 patients with UA and were not predictive for RA development (OR 1.2, 95% CI 0.8 to 2.0, PPV 48%, Table 6.1). RA-specific erosions were present in only 7% of the 2010-

UA patients and were also not associated with development of RA (OR 0.6, 95% CI 0.2 to 1.5, PPV 33%). Similar findings were obtained for the individual 'RA-specific erosions' (Table 6.1). Any MRI-detected wrist erosions were present in 61% of the patients with UA and were also not predictive for RA development (OR 1.5, 95% CI 0.9 to 2.4, PPV 49%). Sensitivity analyses stratified for the outcome (DMARD start or only 2010 criteria positive) revealed similar results (data not shown).

This is the largest longitudinal dataset on MRI-detected erosions in hand and foot joints in UA to date. In all analyses, MRI-detected erosions were not associated with an increased risk on RA. Although MRI is sensitive to detect the presence of erosions, the present data suggest that evaluation of MRI-detected erosions in UA is not relevant for the early detection of RA.

Table 6.1 Predictive values of MRI-detected erosions within 2010-UA patients for the development of RA

	Patients with UA with erosion feature n (%)	PPV (95% CI)	NPV (95% CI)	OR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Any MRI-detected erosion in MCP and/or MTP joint	141 (49)	48% (39-56)	58% (50-66)	1.2 (0.8-2.0)	52% (44-61)	53% (45-61)
Any MRI-detected erosion in the wrist	175 (61)	49% (41-56)	61% (52-70)	1.5 (0.9-2.4)	66% (58-74)	43% (36-51)
Any 'RA-specific erosion'	21 (7)	33% (17-55)	54% (48-60)	0.6 (0.2-1.5)	5% (3-11)	91% (86-95)
Grade ≥2 erosion in MCP and/or MTP joint	5 (2)	40% (12-77)	55% (49-61)	0.8 (0.1-5.0)	2% (0-6)	98% (95-99)
Erosion in MTP5	16 (6)	44% (23-67)	55% (49-61)	1.0 (0.3-2.6)	5% (3-11)	94% (90-97)
Erosion in MTP1 and aged <40	2 (1)	0% (0-66)	55% (49-61)	Undefined	0% (0-3)	99% (96-100)

The prior risk for development of RA and/or DMARD use within 1 year was 45%. Any MRI-detected erosion was defined as score ≥1 by both readers according to the RA MRI Scoring System. Any RA-specific erosion was defined on MRI as the presence of a grade ≥2 erosion in an MCP and/or MTP joint, an erosion in MTP5 and/or an erosion in MTP1 in the age group <40 years, as described earlier (Boeters et al. ARD 2018). CI, confidence interval; DMARD, disease-modifying antirheumatic drug; NPV, negative predictive value; PPV, positive predictive value; RA, rheumatoid arthritis.

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Supplementary material

Supplementary table 6.1 Baseline characteristics of the total group of patients with undifferentiated arthritis and the subgroups of patients with and without progression to rheumatoid arthritis

	All patients with 2010-UA (n=286)	Subgroup of UA patients with progression to RA (n=128)	Subgroup of UA patients without progression to RA (n=158)
Age in years, mean (SD)	55 (16)	58 (16)	52 (15)
Female, n(%)	174 (61)	72 (56)	102 (65)
Symptom duration in weeks, median (IQR)	8 (4-21)	10 (5-25)	7 (3-16)
66-SJC, median (IQR)	2 (1-4)	3 (2-6)	2 (1-3)
68-TJC, median (IQR)	3 (1-7)	5 (2-8)	2 (1-5)
CRP (mg/ml), median (IQR)	4 (3-11)	7 (3-16)	3 (3-6)
RF positivity, n(%)	32 (11)	18 (14)	14 (9)
ACPA positivity, n(%)	24 (8)	20 (16)	4 (3)
Any MRI-detected erosion	152 (53)	71 (55)	81 (51)
Any RA-specific erosion	21 (7)	7 (5)	14 (9)
Grade ≥2	5 (2)	2 (2)	3 (2)
Erosion in MTP5	16 (6)	7 (5)	9 (6)
Erosion in MTP1 and aged <40	2 (1)	0 (0)	2 (1)

Presented are numbers (percentages). Any MRI-detected erosion was defined as score ≥1 by both readers according to the Rheumatoid Arthritis MRI Scoring System. Any RA-specific erosion was defined on MRI as the presence of a grade ≥2 erosion, an erosion in MTP5 and/or an erosion in MTP1 in the age group <40 years, as described earlier (Boeters et al. ARD 2018). ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; IQR, interquartile range; MRI, magnetic resonance imaging; MTP, metatarsophalangeal joint; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, 66-swollen joint count; SD, standard deviation; TJC, 68-tender joint count; UA, undifferentiated arthritis; DMARD, disease-modifying antirheumatic drug.

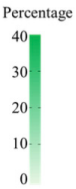
Supplementary table 6.2 Frequencies of RA patients and controls with grade ≥2 erosions and with erosions with the simultaneous presence of local bone marrow edema in the wrist

				Grade ≥2 erosions		Erosions with BME	
			Nr. of persons with erosions	No grade ≥2 erosions	grade ≥2 erosions	Erosion+ BME-	Erosion+ BME+
RA	<40 years	(n=33)	8	7 (87.5%)	1 (12.5%)	6 (75%)	2 (25%)
	40-59 years	(n=96)	60	57 (95%)	3 (5%)	38 (63%)	22 (37%)
	≥60 years	(n=109)	91	84 (92%)	7 (8%)	34 (37%)	57 (63%)
		238					
Other arthritides	<40 years	(n=68)	16	15 (94%)	1 (6%)	11 (69%)	5 (31%)
	40-59 years	(n=146)	95	90 (95%)	5 (5%)	64 (67%)	31 (33%)
	≥60 years	(n=137)	113	97 (86%)	16 (14%)	53 (47%)	60 (53%)
		351					

The presence of grade ≥2 erosions and erosions with BME was evaluated per wrist bone according to the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring system. Grade ≥2 indicates that >10% of the bone is eroded. The presence of BME was defined as a score of ≥1 by both readers. BME, bone marrow edema; RA, rheumatoid arthritis.

Supplementary table 6.3 Location of erosions in bones of the wrist of RA patients and patients with other arthritides, depicted per age category (18-39, 40-59, ≥60 years)

Erosions	<40 years		40-59 years		≥60 years	
	RA	other arthritides	RA	other arthritides	RA	other arthritides
	n=33	n=68	n=96	n=146	n=109	n=137
basis metacarpal 1	3	3	6	6	23	27
basis metacarpal 2	0	0	2	2	9	7
basis metacarpal 3	3	0	3	2	3	4
basis metacarpal 4	0	0	1	1	6	4
basis metacarpal 5	0	0	1	2	1	1
hamate	6	0	8	10	19	17
capitate	9	6	21	23	36	36
trapezoid	6	6	8	10	12	18
trapezium	3	0	5	9	24	28
pisiform	3	0	4	5	6	4
triquetrum	6	3	16	19	27	27
lunate	0	6	13	21	27	28
scaphoid	0	4	18	16	29	35
distal ulna	3	3	7	11	23	18
distal radius	3	0	5	3	10	9



Values are the percentages of persons with an erosion of all persons in that age category. The presence of an erosion is defined as a score of ≥1 in that bone by both readers. RA, rheumatoid arthritis.

PART II

Clinical and imaging features and the ACPA response

7

MRI-detected osteitis is not associated with the presence or level of ACPA alone, but with the combined presence of ACPA and RF

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Arthritis Res Ther. 2016;18:179.

Abstract

Background

In rheumatoid arthritis (RA) bone marrow edema (BME, osteitis) and anti-citrullinated protein antibodies (ACPA) are associated with radiographic progression. ACPA have been associated with BME, but it is unknown if this association is confined to ACPA and BME. We performed cross-sectional analysis of the association of ACPA, rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies with BME and other types of inflammation (synovitis, tenosynovitis) detected by magnetic resonance imaging (MRI).

Methods

Disease-modifying antirheumatic drug (DMARD)-naïve patients with early arthritis (n=589), included in the Leiden Early Arthritis Clinic cohort, underwent contrast-enhanced 1.5T MRI of unilateral wrist, metacarpophalangeal and metatarsophalangeal joints at baseline. BME, synovitis and tenosynovitis were scored by two readers. ACPA, RF and anti-CarP antibodies were determined at baseline.

Results

In univariable analyses ACPA-positive patients had higher BME scores than ACPA-negative patients (median 4.5 vs. 2.0, $p<0.001$), but not more synovitis and tenosynovitis. Also RF (median 3.75 vs. 2.0, $p<0.001$) and anti-CarP antibodies (median 3.5 vs. 2.5, $p=0.012$) were associated with higher BME scores. Because the autoantibodies were concomitantly present, analyses were stratified for the presence of different autoantibody combinations. ACPA+ RF- anti-CarP- patients did not have higher BME scores than ACPA- RF- anti-CarP- patients. However ACPA+ RF+ anti-CarP- patients and ACPA+ RF+ anti-CarP+ patients had higher BME scores than ACPA- RF- anti-CarP- patients (median 5.0 and 4.5 vs. 2.0, $p<0.001$ and $p<0.001$). ACPA levels were not associated with BME scores. Analyses within RA and UA patients revealed similar results.

Conclusions

The presence of ACPA alone or ACPA level was not statistically significantly associated with BME scores, but the combined presence of ACPA and RF was associated with more BME. This suggests an additive role of RF to ACPA in mediating osteitis.

Background

Rheumatoid arthritis (RA) is characterized by chronic inflammation of the joints that may result in progressive structural damage. Magnetic resonance imaging (MRI) detects inflammation sensitively.[1] Whereas synovitis and tenosynovitis can also be evaluated by other imaging modalities, such as ultrasound, MRI is the only modality that depicts bone marrow edema (BME). Histopathology studies in RA have shown that BME lesions consist of infiltration by leucocytes and an increased number of osteoclasts.[2-4] Therefore, BME has also been named osteitis. These data suggest a link between BME and structural damage in RA. Indeed, the importance of BME is supported by several studies showing that BME is a predictor of radiographic evidence of progression.[5-13] A recent study even showed that the persisting presence of BME is associated with an odds ratio of 60 for erosive progression at the same location.[14]

In addition to BME, anti-citrullinated protein antibodies (ACPA) are also a strong predictor of radiographic progression.[15-23] Up to two-thirds of patients with RA harbor ACPA, as has been known for many years.[24] However, the underlying mechanism linking ACPA with a more severe disease progression with increased joint destruction is incompletely elucidated. Recent data suggest that ACPA influences bone resorption by directly activating osteoclasts.[25] The combination of these findings lead us to hypothesize that ACPA are associated with BME.

Other studies, including a small study that we performed previously suggest there is association between BME and ACPA.[26,27] However, ACPA are often simultaneously present with other RA-related autoantibodies such as rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies (which also have been associated with radiographic destruction).[28-30] To our knowledge the effects of different autoantibodies (either alone or in combination) on BME are unknown. Furthermore, the association between different autoantibodies and other types of inflammation detected by MRI (synovitis and tenosynovitis) has never been thoroughly explored. Therefore, this cross-sectional study aimed to investigate the associations of ACPA, RF and anti-CarP antibodies with BME, synovitis and tenosynovitis.

Methods

Patients

The 589 patients with early arthritis were consecutively included in the Leiden Early Arthritis Clinic (EAC) between 2010 and 2014. The EAC is an inception cohort that includes patients attending the rheumatologist who present with clinically confirmed arthritis and symptom duration of <2 years. Patients were disease-modifying antirheumatic drug (DMARD)-naïve at inclusion. The cohort started in 1993. MRI was performed from 2010 onwards; 598 patients underwent MRI, and 9 were excluded from analysis because no contrast agent was administered. The median interval between inclusion in the study and MRI was 1.3 weeks. Questionnaires were administered, and joint counts and blood samples were collected at baseline.[31] Baseline serum samples were tested for ACPA (anti-CCP2, Eurodiagnostica, Arnhem, the Netherlands, cut-off value ≥7 U/ml), IgM RF (as described previously, in-house ELISA[32]) and IgG anti-CarP antibodies against carbamylated fetal calf serum (FCS). Anti-CarP antibodies were determined as described previously;[28] the cut-off for positivity for anti-CarP antibodies was based on the mean plus two times the standard deviation from a set of 204 healthy controls. One year after presentation, 183 patients fulfilled the 1987 criteria for RA,[33] 214 had undifferentiated arthritis (UA) and 192 had other forms of arthritis, including psoriatic arthritis, reactive arthritis and others (Table 7.1).

Magnetic resonance imaging and scoring

At baseline, MRI was performed of the metacarpophalangeal (MCP), wrist and metatarsophalangeal (MTP) joints on the most painful side or on the dominant side in the case of symmetric symptoms. MRI was performed using an MSK Extreme 1.5T extremity MRI system. In the wrist and MCP joints a coronal T1-weighted sequence was acquired before intravenous injection of contrast agent (gadoteric acid). Post-contrast, coronal and axial T1-weighted sequences with frequency-selective fat saturation were obtained. The forefoot was scanned using a T1-weighted sequence and a T2-weighted fat-saturated sequence in the axial plane. The protocol was shortened after 371 patients had been imaged.[34] For post-contrast imaging of the foot in the remaining 218 patients, T1-weighted, fat-saturated sequences were obtained in the coronal and axial plane and the T2-weighted sequence was deleted. A more detailed description of the scan protocol is provided elsewhere[14,35,36] and in the online Supplementary methods.

BME and synovitis were scored semi-quantitatively according to the rheumatoid

arthritis MRI scoring system (RAMRIS),[37] with the exception that BME was assessed on a contrast-enhanced T1-weigthed fat-suppressed sequence. Previous studies have shown that T2-weighted fat-saturated sequences and contrast-enhanced T1-weighted fat-saturated images perform equally well in the depiction of BME,[34,38,39] and according to the European Society of Musculoskeletal Radiology (ESSR), both sequences can be used to evaluate BME.[40] The T1-sequence was used as it allowed a shorter scan time. In addition, tenosynovitis in the wrist and MCP joints was scored according to the method proposed by Haavardsholm et al.,[41] with tenosynovitis assessed for the flexor and extensor tendons of MCP joints 2-5 using the same scale of 0-3 as for the wrist. MR images were scored by two readers blinded to any clinical data. The mean total scores of both readers for BME, synovitis and tenosynovitis were used in further analyses. The intra-reader class correlation coefficients for the total inflammation score based on 40 MR images that were scored twice, were 0.98 and 0.93, respectively. Based on all 598 scans, the interreader class correlation coefficient for the total inflammation score was 0.95.

Sensitivity analyses

Our primary analyses were performed in all 589 early arthritis patients, as we hypothesized that direct association between autoantibodies and MRI-detected inflammation, if present, would be independent of the clinical diagnosis. However, analyses were repeated in the subgroup of 397 patients classified with UA or RA according to the 1987 American College of Rheumatology (ACR) criteria. Patients who had UA after one year were included in these analyses because misclassification could have occurred due to DMARD treatment during the first year that could have hampered progression to fulfilling the 1987 criteria for RA. As some of these patients with UA would have progressed to RA without treatment (but now remain unclassified), we also studied the patients with UA.

Statistical analysis

The t test, multivariable linear regression, and multivariable logistic regression were used for analysis as appropriate. In multivariable linear regression analysis, the BME scores were log10-transformed (log10(score+1)) to approximate a normal distribution. For interpretation, the obtained effect size (beta) was back-transformed to the normal score. All models were adjusted for age, gender and symptom duration. Baseline data on ACPA, RF and anti-CarP antibodies were dichotomized (seropositive vs. seronegative). Anti-CarP data were missing for 16 patients. ACPA and RF status was known for all patients. To determine the effect of ACPA levels on BME, baseline ACPA was categorized into three groups within

ACPA-positive patients based on the range of ACPA values (low, intermediate, or high); the thresholds were: ≥7 U/ml, ≥167 U/ml and ≥327 U/ml. P-values <0.05 were considered significant. Analyses were performed using SPSS version 23.0 (IBM).

Results

Baseline characteristics

Baseline characteristics of the 589 patients are presented in Table 7.1.

Table 7.1 Baseline characteristics of the total group of early arthritis patients studied and the subgroups of patients with RA or UA

Variable	All patients with early arthritis (n=589)	Subgroup of patients with RA or UA (n=397)
Age, mean (sd)	54.8 (16)	54.9 (15)
Female, n (%)	363 (62)	253 (64)
Symptom duration, median (IQR), weeks	12.6 (5-27)	12.2 (5-26.2)
TJC, median (IQR)	4 (2-7)	4 (2-6)
SJC, median (IQR)	3 (2-7)	3 (2-7)
CRP (mg/L), median (IQR)	5.7 (3-17)	6 (3-17)
ACPA positivity, n (%)	141 (24)	123 (31)
RF positivity, n (%)	193 (33)	151 (38)
Anti-CarP positivity, n (%)	88 (15)	71 (18)
Total RAMRIS, median (IQR)	12.5 (5.5-24)	13.5 (6-24)
Total BME score, median (IQR)	2.5 (1-6)	2.5 (1-6)
Total synovitis score, median (IQR)	3.5 (1-7.5)	4 (1.5-8)
Total tenosynovitis score, median (IQR)	2 (0-6)	3 (0.5-6)

The diagnoses of the 589 patients with early arthritis were: 183 RA (according to the 1987 RA criteria), 214 UA, 14 reactive arthritis, 14 gout, 2 pseudogout, 30 psoriatic arthritis, 35 inflammatory osteoarthritis, 4 Lyme's arthritis, 1 paramalignant arthritis, 3 systemic lupus erythematosus, 11 other systemic disorder, 7 mixed connective tissue disease, vasculitis, 4 sarcoidosis, 9 spondyloarthritis, 8 remitting seronegative symmetrical synovitis with pitting edema, and 50 other unspecified conditions. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CRP, C-reactive protein; IQR, interquartile range; n, number of patients; RA/UA, subgroup of patients with rheumatoid arthritis (according to the 1987 RA criteria) or undifferentiated arthritis; RF, rheumatoid factor; sd, standard deviation; SJC, 66-swollen joint count; symptom duration, time between symptom onset and inclusion in cohort; TJC, 68-tender joint count.

ACPA is associated with BME at baseline

We first evaluated whether patients with ACPA (n=141) or without ACPA (n=448) had differences in BME scores (Figure 7.1A). ACPA-positive patients had higher BME scores (median=4.5) than ACPA-negative patients (median=2.0, p<0.001). We

subsequently questioned whether ACPA is also associated with other types of MRI-detected inflammation, i.e., synovitis and tenosynovitis. There were no statistically significant differences in synovitis or tenosynovitis scores in ACPA-positive and ACPA-negative patients (Figure 7.1A). Similar results were obtained for BME when only patients with RA and UA were studied (ACPA-positive median=3.5, ACPA-negative median=2.0, p=0.001) and no statistically significant differences were observed for synovitis and tenosynovitis (Supplementary figure 7.1A). Based on these data ACPA seemed to be primarily associated with BME.

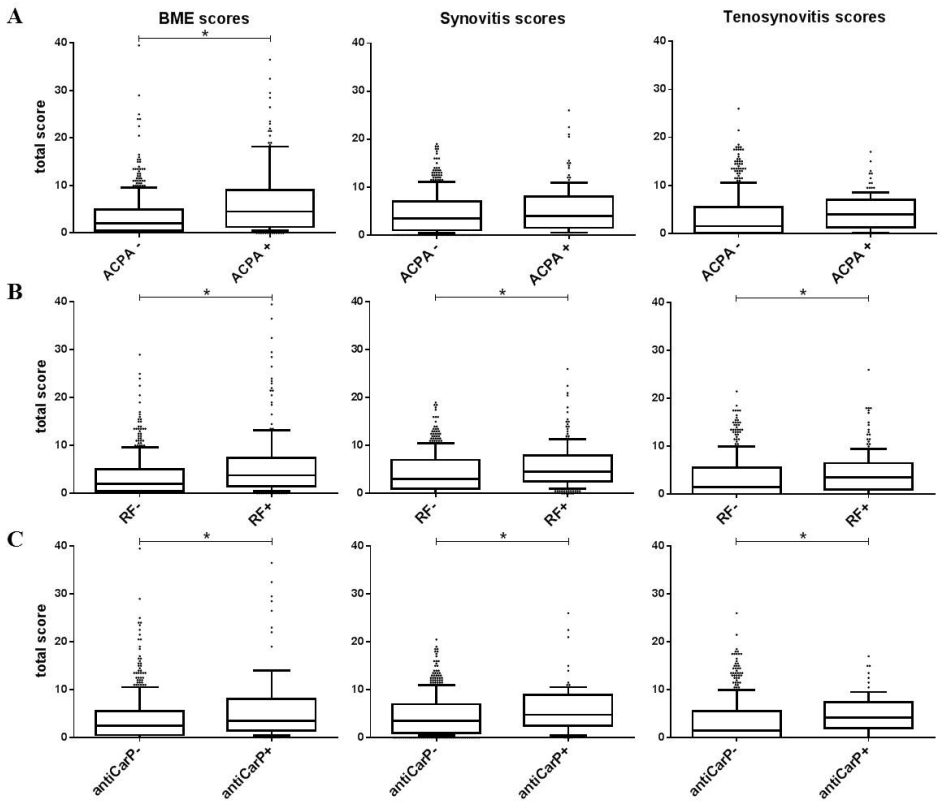

Figure 7.1 Illustration of the association between anti-citrullinated protein antibodies (ACPA) (A), rheumatoid factor (RF) (B) and anti-carbamylated protein antibodies (anti-CarP) (C), and magnetic-resonance-imaging-detected bone marrow edema (BME), synovitis and tenosynovitis scores in early arthritis (n=589)

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. (A) BME: p≤0.001; synovitis: p=0.084; tenosynovitis: p=0.064. (B) BME: p≤0.001; synovitis: p=0.001; tenosynovitis: p=0.004. (C) BME: p≤0.012; synovitis: p=0.021; tenosynovitis: p=0.013. Total score: sum of scores in metacarpophalangeal, wrist, and metatarsophalangeal joints. *Significant difference (p<0.05) between autoantibody-negative and autoantibody-positive patients.

RF and anti-CarP antibodies are also associated with BME

We were also interested in whether RF and anti-CarP antibodies are also associated with more severe BME. The BME scores were higher in RF-positive patients (median=3.75) compared to RF-negative patients (median=2.0, p<0.001, Figure 7.1B). Similarly, BME scores were also higher in anti-CarP-positive than in anti-CarP-negative patients (median=3.5 vs. 2.5, p=0.012, Figure 7.1C). Besides BME, synovitis and tenosynovitis scores were also higher in RF-positive than in RF-negative patients (synovitis: median 4.5 vs. 3.0, p=0.001; tenosynovitis: median 3.5 vs. 1.5, p=0.004). Synovitis and tenosynovitis scores were also higher in anti-CarP-positive than in anti-CarP-negative patients (synovitis: median=4.75 vs. 3.5, p=0.021; tenosynovitis: median=4.25 vs. 1.5, p=0.013). In patients with RA and UA only the BME scores were significantly higher in RF-positive (RF+) or anti-CarP-positive (anti-CarP+) patients, but synovitis and tenosynovitis scores were not statistically significantly different (BME: RF+ median=3.5, RF-negative (RF-) median=2.0, p=0.002; anti-CarP+ median=3.5, anti-CarP-negative (anti-CarP-) median=2.5, p=0.017, Supplementary figure 7.1B, C).

Patients can concurrently have BME, synovitis, and tenosynovitis. To unravel the independent association between RF and BME, synovitis, and tenosynovitis scores, multivariable logistic regression analysis was performed with RF as the dependent variable and BME, synovitis, and tenosynovitis as independent variables. The same was done with anti-CarP antibodies as the dependent variable. In early arthritis, only the BME score was independently associated with RF (p<0.001) or with anti-CarP antibodies (p=0.003). Similar results were observed in the subgroup of patients with RA or UA, in whom only BME was associated with RF (p<0.001) or with anti-CarP antibodies (p=0.001). Thus, these multivariable analyses suggest that the BME score is independently associated with RF or anti-CarP antibodies, in contrast to the synovitis and tenosynovitis scores. Because of this result, and because it was observed that there was an association between BME and ACPA, subsequent analyses focused on BME.

ACPA and RF are both independently associated with BME

Patients frequently have a combination of different types of inflammation, and also concomitantly have the three autoantibodies. For more insight into the relationship between the different autoantibodies and BME, multivariable linear regression analysis was performed with BME as the outcome and the three autoantibodies as independent variables. Both ACPA and RF were significantly associated with BME (ACPA: p=0.015, beta=1.33, indicating that ACPA-positive (ACPA+) patients

had 33% higher BME scores than ACPA-negative (ACPA-) patients; RF: p=0.004, beta=1.31, indicating that RF+ patients had 31% higher BME scores than RF- patients). Additional adjustments for CRP and SJC produced similar results (ACPA: p=0.009, beta=1.36; RF: p=0.001, beta=1.36). In a similar analysis in the subgroup of patients with RA and UA, there was a trend towards significance for ACPA (p=0.091, beta=1.26) and a significant result for RF (RF: p=0.022, beta=1.31). Thus, together these data indicated that ACPA and RF are independently associated with BME scores.

Combined presence of ACPA and RF is associated with BME

The multivariable analyses described above did not evaluate different effects for combinations of antibodies. Different autoantibody combinations were compared for more insight into the effect of individual antibodies and a combination of antibodies on BME (Figure 7.2). In the absence of both RF and anti-CarP antibodies, ACPA was not associated with BME (ACPA+ RF- anti-CarP- patients vs. ACPA- RF- anti-CarP- patients, median 1.0 vs. 2.0, p=0.43). Also the presence of RF or anti-CarP antibodies alone was not associated with BME (ACPA- RF+ anti-CarP- patients and ACPA- RF- anti-CarP+ patients vs. ACPA- RF- anti-CarP- patients, median 2.5 and 1.5 vs. 2.0 respectively, p=0.096 and p=0.43). However ACPA+ RF+ anti-CarP- patients and ACPA+ RF+ anti-CarP+ patients did have significantly higher BME scores than ACPA- RF- anti-CarP- patients (median 5.0 and 4.5 vs. 2.0 respectively, p<0.001 and p<0.001). The same analysis in only RA and UA patients showed that ACPA+ RF+ anti-CarP- patients and ACPA+ RF+ anti-CarP+ patients had higher BME scores than ACPA- RF- anti-CarP- patients (median 4.5 and 4.5 vs. 2.0 respectively, p<0.001 and p<0.001, Figure 7.3). Thus only the combined presence of ACPA and RF (with or without the presence of anti-CarP antibodies) was associated with higher BME scores.

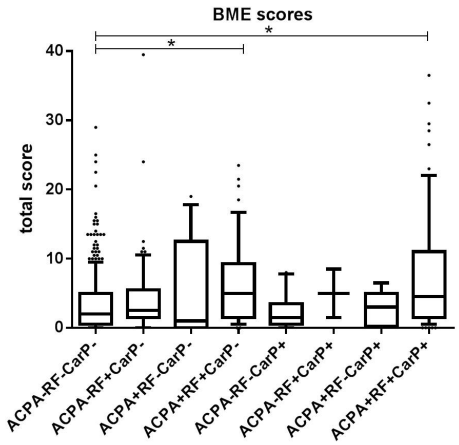

Figure 7.2 Bone marrow edema (BME) scores in patients with early arthritis (n=589) with different combinations of anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF) and anti-carbamylated protein antibodies (anti-CarP)

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. ACPA+ RF+ anti-CarP- patients vs. ACPA- RF- anti-CarP- patients, p<0.001; ACPA+ RF+ anti-CarP+ patients vs. ACPA- RF- anti-CarP- patients, p<0.001. ACPA- RF- anti-CarP- patients, n=353; ACPA- RF+ anti-CarP- patients, n=68; ACPA+ RF- anti-CarP- patients, n=15; ACPA+ RF+ anti-CarP- patients, n=48; ACPA- RF- anti-CarP+ patients, n=11; ACPA- RF+ anti-CarP+ patients, n=3; ACPA+ RF- anti-CarP+ patients, n=5; ACPA+ RF+ anti-CarP+ patients, n=69. Total score: sum of BME scores in metacarpophalangeal, wrist, and metatarsophalangeal joints. *Significant difference (p<0.05) between subgroups.

ACPA level is not associated with BME

In general, patients who carry different RA-related autoantibodies also have higher levels of ACPA.[42] In our present data we also observed higher ACPA levels in patients who also carried RF and anti-CarP antibodies (ACPA+ RF- anti-CarP- patients median 116 U/ml, ACPA+ RF+ anti-CarP- patients median 155 U/ml, ACPA+ RF- anti-CarP+ patients median 92 U/ml, ACPA+ RF+ anti-CarP+ patients median 257 U/ml, p=0.020). This prompted us to explore whether the combined presence of ACPA and RF with higher BME scores could be explained by higher ACPA levels. To investigate the association between BME and ACPA levels, ACPA were studied as continuous data (Supplementary figure 7.2) and divided into three subgroups. The BME scores observed in these ACPA categories were not different (Figure 7.4). Similarly, no differences were observed when analyzing the BME scores in relation to ACPA levels in patients with RA and UA (Supplementary figure 7.3). These data suggest that it is the combined presence of ACPA and RF that is associated with BME, rather than ACPA levels.

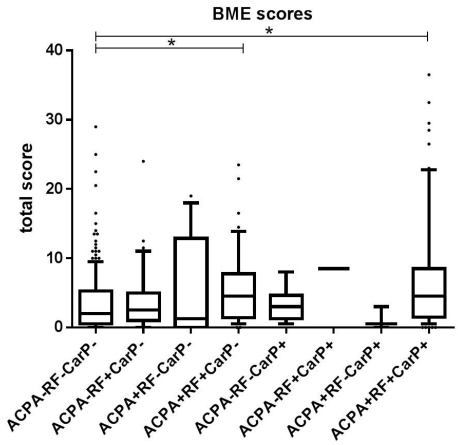

Figure 7.3 Bone marrow edema (BME) scores in patients with rheumatoid arthritis (RA) and undifferentiated arthritis (UA) (n=397) with different combinations of anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF) and anti-carbamylated protein antibodies (anti-CarP)

Horizontal lines representing median. Whiskers show the 10th-90th percentile. Dots represent outliers. ACPA+ RF+ anti-CarP+ patients vs. ACPA- RF- anti-CarP- patients, p<0.001; ACPA+ RF+ anti-CarP- patients vs. ACPA- RF- anti-CarP- patients, p<0.001. ACPA-, RF-, anti-CarP- patients, n=217; ACPA- RF+ anti-CarP- patients, n=43; ACPA+ RF- anti-CarP- patients, n=14; ACPA+ RF+ anti-CarP- patients, n=42; ACPA- RF- anti-CarP+ patients, n=6; ACPA- RF+ anti-CarP+ patients, n=1; ACPA+ RF- anti-CarP+ patients, n=3; ACPA+ RF+ anti-CarP+ patients, n=61. Total score: sum of BME scores in metacarpophalangeal, wrist, and metatarsophalangeal joints. *Significant difference between subgroups (p<0.05).

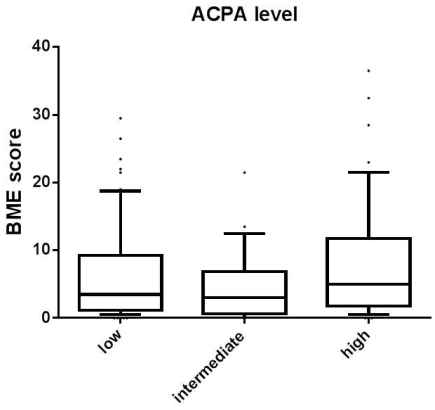

Figure 7.4 Bone marrow edema (BME) scores in anti-citrullinated protein antibodies (ACPA)-positive patients with early arthritis (n=141) with low, intermediate, or high levels of ACPA

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. Baseline ACPA is shown categorically as low, intermediate, or high. The groups were as follows: low ≥7 U/ml, intermediate ≥167 U/ml and high ≥327 U/ml. Low: n=64; intermediate: n=32; high: n=45. Kruskal-Wallis test, p=0.14.

Combined presence of ACPA, RF and anti-CarP antibodies is associated with synovitis and tenosynovitis

The analyses focused on BME as the different autoantibodies were not associated with synovitis or tenosynovitis scores in univariable analyses (for ACPA) or in multivariable analyses (for RF and anti-CarP antibodies). However, having observed that higher BME scores were primarily associated with the combined presence of ACPA and RF (and not with the presence of a single antibody), we reasoned that it might also be possible that antibodies were not individually associated with synovitis or tenosynovitis scores, but that some combinations of autoantibodies were associated with synovitis or tenosynovitis scores. To study this, we finally assessed the association between different combinations of autoantibodies and synovitis and tenosynovitis (Figure 7.5). ACPA+ RF+ anti-CarP+ patients had higher synovitis scores than ACPA- RF- anti-CarP- patients (median 5.0 vs. 3.0, p=0.001). For tenosynovitis, ACPA+ RF+ anti-CarP+ patients had higher scores than ACPA- RF- anti-CarP- patients (median 4.5 vs. 1.0, p<0.001), and ACPA+ RF+ anti-CarP+ patients had higher scores than ACPA+ RF+ anti-CarP- patients (median 4.5 vs. 3.5 p=0.039). Thus, the combined presence of ACPA, RF and anti-CarP antibodies was associated with the highest synovitis and tenosynovitis scores.

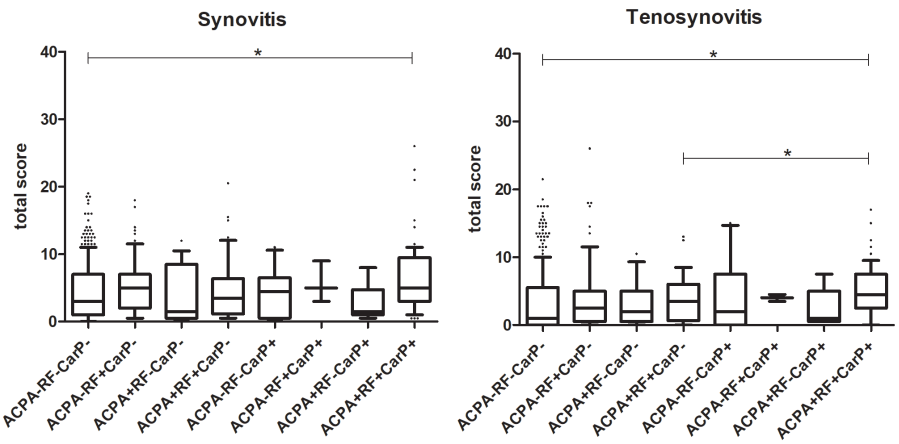

Figure 7.5 Scores for synovitis detected by magnetic resonance imaging (A) and tenosynovitis (B) in patients with early arthritis (n=589) with different combinations of anti-citrullinated protein antibodies (ACPA), rheumatoid factor (RF) and anti-carbamylated protein antibodies (anti-CarP)

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. Synovitis: ACPA+ RF+ anti-CarP+ patients vs. ACPA- RF- anti-CarP- patients, p<0.001. Tenosynovitis: ACPA+ RF+ anti-CarP+ patients vs. ACPA- RF- anti-CarP- patients, p<0.001; ACPA+ RF+ anti-CarP+ patients vs. ACPA+ RF+ anti-CarP- patients, p=0.039. ACPA- RF- anti-CarP- patients, n=353; ACPA- RF+ anti-CarP- patients, n=69; ACPA+ RF- anti-CarP- patients, n=15; ACPA+ RF+ anti-CarP- patients, n=48; ACPA- RF- anti-CarP+ patients, n=11; ACPA- RF+ anti-CarP+ patients, n=3; ACPA+ RF- anti-CarP+ patients, n=5; ACPA+ RF+ anti-CarP+ patients, n=69. Total score: sum of scores in metacarpophalangeal, wrist, and metatarsophalangeal joints. *Significant difference between subgroups (p<0.05).

Discussion

The relationship between ACPA and other RA-related autoantibodies and BME was subject of this study. We showed that ACPA, RF and anti-CarP antibodies were all associated with BME in univariable analyses. However, when the different autoantibody combinations were compared, the presence of ACPA alone was not associated with BME, but the combined presence of ACPA and RF (with or without anti-CarP antibodies) was associated with BME. The level of ACPA was not associated with BME, suggesting that this cannot be explained by ACPA levels but rather by the combined presence of ACPA and RF.

To our knowledge this is the first study including almost 600 MR images in which the relationship between the different autoantibodies and MRI-detected inflammation was investigated in detail. Due to this large sample size it was possible to evaluate the independent associations between BME and ACPA, RF and anti-CarP antibodies. Furthermore, it was possible to investigate the differential effects of the autoantibodies on the different types of MRI-detected inflammation. On analyses in subgroups of patients with different autoantibody combinations the BME scores were mainly increased when both ACPA and RF were present.

Our data suggest a potential interaction between RF and ACPA; however, the underlying mechanism by which ACPA and RF could act in concert was not studied. Potentially RF could have an immune-enhancing effect by crosslinking immune complexes and thereby activate monocytes or macrophages and induce cytokine expression. This is supported by a recent study that showed that RF augments TNFα production by ACPA immune complexes in vitro.[43] Another explanation could be that RF has a role in immune complex stabilization. ACPA bind to target antigens with low avidity but it could well be that when RF is also involved in the immune complex this binding is more stable. Further fundamental studies should be performed for more insight into the interaction between these two autoantibodies.

This study investigated local inflammation as observed on MRI. Recently the combined effect of ACPA and RF on systemic inflammation was investigated in RA, showing that the combined presence of ACPA and RF was associated with higher levels of pro-inflammatory cytokines and increased acute phase reactants and disease activity.[43] We also analyzed the association between the different autoantibody combinations and CRP, erythrocyte sedimentation rate (ESR), SJC and 28-joint count disease activity score (DAS28) as measures of disease activity

in our patients with RA or UA at baseline; no large differences were observed but patients positive for all three autoantibodies had the highest disease activity scores (Supplementary figure 7.4).

Association between the combined presence of autoantibodies and BME was observed. Since BME is associated with erosive progression,[5-14] it would be interesting to investigate whether combinations of autoantibodies are also associated with radiographic progression. The association between ACPA or RF and radiographic progression is well-investigated.[15-23] However the number of studies investigating the combined effect of ACPA and RF is limited. A recent study in two cohorts showed no additive effect of RF on radiographic progression in ACPA-positive patients.[44] Another study analyzing high-resolution peripheral quantitative computed tomography (CT) images of the MCP joints in patients with RA showed that there was an additive effect of ACPA and RF on erosion number and size.[45] The read-out of these studies (microCT and conventional radiography) was different. It would be interesting to further unravel the association between different autoantibody combinations and erosive progression in further observational studies.

A limitation of the subgroup analysis is that some autoantibody combinations were infrequent and so no definite conclusions can be drawn for these. For instance, patients who were ACPA+ RF- anti-CarP- were infrequent. Despite the limited power, there was no tendency in the data towards higher BME scores in these patients compared to the triple-negative group. This study is not the first that did not identify a deleterious effect of the presence of ACPA alone. Two recent papers reported on mice that were injected with ACPA, and although ACPA was detected in the joint, no signs of inflammation were observed in the synovium.[46,47] Surprisingly, in our data the presence of ACPA alone even had a non-significant tendency towards a protective effect against synovitis (in all patients with early arthritis patients and in patients with RA or UA). Interestingly, two recent studies in humans showed that the presence of ACPA without RF was associated with lower disease activity.[42,43] In summary, further larger studies are needed to determine the role of ACPA single positivity.

Another limitation could be that we used contrast-enhanced T1-weighted images to assess BME. Using the RAMRIS method, T2-weighted fat-suppressed sequences, or when this sequence is not available, a short tau inversion recovery (STIR) sequence, should be used to assess BME. However, it has been demonstrated that a contrast-enhanced T1-weighted fat-suppressed sequence performed equally well

to depict BME as a T2-weighted fat-suppressed sequence[34,38,39] and the evaluation of BME on a T1-weighted fat suppressed sequence is also supported by the ESSR.[40] In this study the contrast-enhanced T1-weighted fat-suppressed sequence was used because it allowed a shorter scan time and has a higher signal-to-noise ratio.[34,38]

A third limitation is that the scan protocol for the foot was changed. When the analyses of the different autoantibody combinations and BME scores in patients with early arthritis were repeated separately in the patients scanned with or without the coronal sequence of the foot, the presence of ACPA alone was not associated with higher BME scores, but the combined presence of ACPA and RF was associated with higher BME scores (data not shown). This suggests that the change in scan protocol for the foot had no major influences on the results of this study.

Finally, our arthritis cohort includes patients with early disease who presented with different diagnoses. We hypothesized that direct association between ACPA and MRI-detected inflammation would be independent of the clinical diagnosis. However, to exclude an effect of this heterogeneity in patient selection on our findings we repeated all analyses within the subgroup of patients with RA and UA. This produced similar results.

Of note, the differences observed in BME scores were statistically significant but the absolute differences were relatively small. The variation in BME scores was only partly explained by the autoantibody status. Nonetheless the present study does increase our understanding of the relationship between autoantibodies and BME, which are both predictors of radiographic progression. The observation that ACPA is associated with osteitis, only when RF is present, fuels further laboratory studies on the biological relevance of these autoantibodies.

Conclusions

In conclusion, the presence of ACPA alone and ACPA serum levels were not associated with BME scores. However, BME scores were higher when patients were seropositive for both ACPA and RF. These results suggest that RF has an additive role to ACPA in mediating osteitis.

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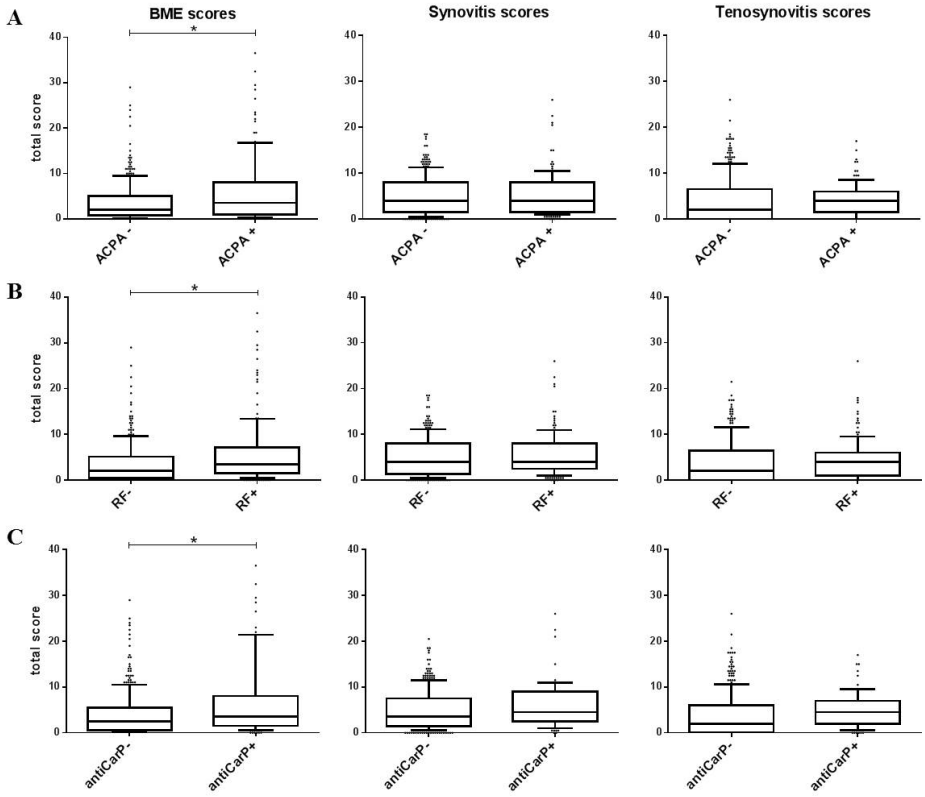
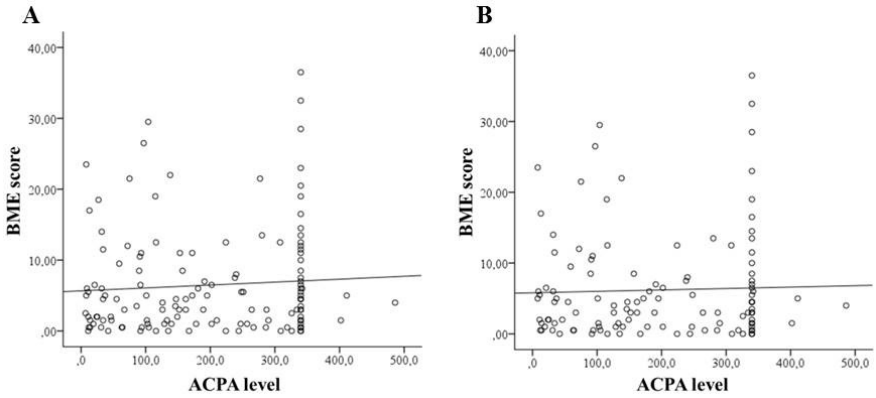
Supplementary material

Supplementary methods are available at the website of Arthritis Research and Therapy.



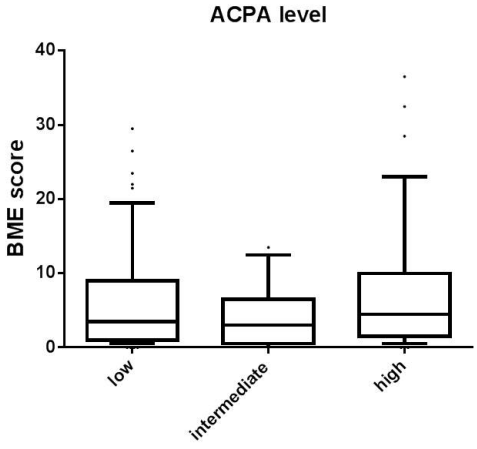
Supplementary figure 7.1A Illustration of association between ACPA (A), RF (B) and anti-CarP antibodies (C) and MRI-detected BME, synovitis and tenosynovitis scores in patients with RA and UA (n=397)

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. (A) BME: p=0.001; synovitis: p=0.776; tenosynovitis: p=0.99. (B) BME: p=0.002; synovitis: p=0.19; tenosynovitis: p=0.26. (C) BME: p=0.017; synovitis: p=0.085; tenosynovitis: p=0.056. Total score: sum of scores in MCP, wrist, and MTP joints. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; BME, bone marrow edema; MRI, magnetic resonance imaging; RF, rheumatoid factor. *Significant difference between autoantibody-negative and autoantibody-positive patients (p<0.05).



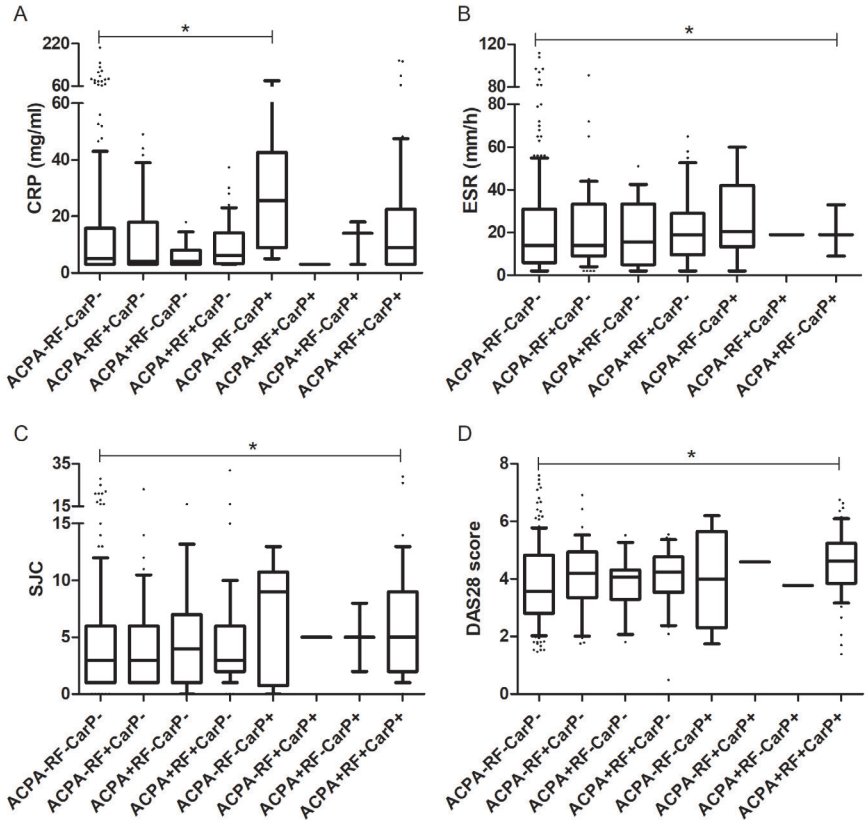
Supplementary figure 7.2 Association between ACPA level and BME scores within ACPA-positive patients with early arthritis (A) (n=141, r=0.071, p=0.403) and within ACPA-positive patients with RA or UA (B) (n=123, r=0.034, p=0.706)

ACPA, anti-citrullinated protein antibodies; BME, bone marrow edema; RA, rheumatoid arthritis; UA, undifferentiated arthritis.



Supplementary figure 7.3 BME scores of ACPA-positive patients with RA or UA (n=123) with low, intermediate, or high levels of ACPA

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. Baseline ACPA levels are shown categorically as low, intermediate, or high. The groups were as follows: low ≥7 U/ml, intermediate ≥167 U/ml, and high ≥327 U/ml. Low: n=57; intermediate: n=27; high: n=39. ACPA, anti-citrullinated protein antibodies; BME, bone marrow edema; RA, rheumatoid arthritis; UA, undifferentiated arthritis. Kruskal-Wallis test, p=0.23.



Supplementary figure 7.4 Association between CRP (A), ESR (B), SJC (C) and DAS28 (D) and different autoantibody combinations

Horizontal lines represent median. Whiskers show the 10th-90th percentile. Dots represent outliers. ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; SJC, swollen joint count based on 66 joints. *Significant difference between subgroups ($p<0.05$).

8

The prevalence of ACPA is lower in rheumatoid arthritis patients with an older age of onset but the composition of the ACPA response appears identical

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Abstract

Background

Rheumatoid arthritis (RA) consists of two syndromes, one autoantibody-positive and one autoantibody-negative. Existing data on the relation between age of onset and prevalence of autoantibodies were conflicting. Therefore this multicohort study assessed the age of onset in relation to the presence of autoantibodies. The association with characteristics of the anti-citrullinated protein antibodies (ACPA) response was also explored.

Methods

The 1987 criteria-positive RA patients included in the Leiden EAC, BARFOT, ESPOIR, Umeå and Lund cohorts (n=3321) were studied at presentation for age of onset and the presence of ACPA, rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies. Logistic regression analyses were performed; effect sizes were summarized in inverse-weighted meta-analyses. Within ACPA-positive RA, ACPA level was studied in all cohorts; ACPA isotypes, ACPA fine specificity and ACPA avidity index and clinical characteristics were studied in the Leiden EAC.

Results

From the age of 50 onward, the proportion of ACPA-negative RA patients increased with age in the five cohorts. Similar observations were made for RF and anti-CarP antibodies. The composition of the ACPA response did not change with increasing age of onset with respect to titer, isotype distribution, fine specificity and avidity index. With increasing age of onset, RA patients smoked less often, had higher acute phase reactants and more often had a sub(acute) symptom onset.

Conclusions

Data of five cohorts revealed that with older age of onset ACPA-negative RA is more frequent than ACPA-positive RA, while characteristics of ACPA-positive RA as judged by the composition of the ACPA response appeared not age dependent. Further biologic studies are needed to characterize the pathogenesis of ACPA-negative polyarthritis at older age and to promote personalized treatment decisions in ACPA-negative patients in daily practice.

Background

Rheumatoid arthritis (RA) is a syndrome for which characterization is based on a combination of clinical features. Symmetric polyarthritis of hands and feet is a key clinical feature and is captured in the 1987 classification criteria.[1] It is presumed that different biologic pathways can end up in the same clinical phenotype of RA. To arrive at personalized medicine, it is relevant to identify such different groups of patients. The most commonly used division is that into anti-citrullinated protein antibodies (ACPA)-positive RA and ACPA-negative RA, and both subgroups have differences in genetic and environmental risk factors.[2,3]

In addition to ACPA, there is some evidence suggesting that there are different characteristics of RA patients presenting at an older age. Several studies have shown that RA patients with disease onset at older age have a more equal gender distribution, more frequently an acute onset of symptoms[4,5] and more often experience constitutional symptoms than younger patients at disease presentation.[4-6] ACPA positivity is more frequent with older age, suggesting that ACPA-positive RA may also be more frequent with older age.[7] However, within autoantibody-positive patients it was recently observed that patients with two or three autoantibodies were younger at onset than patients with one autoantibody.[8] In addition, some studies showed lower frequencies of autoantibodies in RA patients presenting at older age,[9-14] while other studies observed no differences[15,16] or showed a nonsignificant trend toward a higher prevalence of ACPA in RA patients presenting at older age.[17,18] Altogether, the association between age of onset and the distribution of ACPA-positive RA versus ACPA-negative RA remains to be established.

If there is an association between age of onset and the presence of autoantibodies, this could be explained by different scenarios. There could be an age-related effect on the ACPA response itself. Then, in addition to the presence of ACPA, characteristics of the ACPA response could also vary with age. This could be a conceivable explanation because in the general population the immune system changes with ageing. For instance, an increase in proinflammatory cytokines, a more active innate immunity and a decline in the function of the adaptive immune system has been observed with older age.[19] T cell senescence has been described and may mediate the development of RA.[20] With regard to B cells and antibodies, titers of antibodies against nearly all vaccines, including tetanus, decrease with age.[21] Furthermore there is a defect in isotype switching and limited production of

high-affinity antibodies with increasing age, all thought to associate with decreased protection by vaccines and increased susceptibility to infections.[22] Whether B cell senescence has a role in RA development is still unclear.[19] Despite these studies on the autoantibody response and aging, to our knowledge it is unknown whether age influences characteristics of the ACPA response, measured at RA onset.

An alternative explanation could be that some of the patients presenting at older age with 'typical RA' (e.g., symmetric polyarthritis of small joints) have differences in underlying biologic mechanisms compared with younger patients. Although biologic studies are needed to verify this hypothesis, detailed phenotypic studies can identify subtle differences between patient groups, despite their similarity in key clinical characteristics that are required for classification.

As a follow-up on previous studies of ACPA and age of onset as well as on the mentioned considerations, this study had three aims. Firstly, to determine the association between age of RA onset and the frequency of three autoantibodies (ACPA, rheumatoid factor (RF) and anti-carbamylated protein (anti-CarP) antibodies). For this purpose a large study on data of five cohorts was performed. Secondly, to study whether age at onset was associated with characteristics of the ACPA response in ACPA-positive RA patients. Thirdly, to substantiate previously reported associations between age at onset and clinical characteristics.[4-6]

Methods

Patients

The association between age at RA onset and prevalence of ACPA and RF was studied in all five RA cohorts (Leiden Early Arthritis Clinic (EAC), BARFOT, ESPOIR, Umeå, Lund) and anti-CarP antibodies were studied in two cohorts (Leiden EAC, BARFOT). The association between age at RA onset and ACPA level was also studied in all five cohorts. Other ACPA characteristics were studied in ACPA-positive RA patients from the Leiden EAC. RA was defined as fulfilling the 1987 classification criteria.[1] The 2010 classification criteria were not used because autoantibodies are heavily weighted in these criteria, which may induce circularity between the parameter that was studied and the reference.

Leiden EAC

The Leiden EAC is an inception cohort set up in the Leiden University Medical

Center (the Netherlands) that started in 1993 and includes patients with clinically confirmed arthritis and symptom duration <2 years at presentation to the rheumatologist.[23] This department is the only referral center in a health care population of >400,000 inhabitants. At baseline questionnaires, joint counts and blood samples were collected. Information on smoking (present versus none and past) was obtained at baseline. The presence of shared epitope alleles was determined as described previously.[24] The patients studied were included between 1993 and 2015; a total of 1244 RA patients were consecutively included and studied here. The age ranged between 18 and 92 years.

BARFOT

The BARFOT (Better Anti-Rheumatic Farmaco-Therapy) study is an observational study of patients with early RA in Sweden.[25] Patients aged 18-93 years were included from six rheumatology centers when they were diagnosed with RA and had symptom duration <1 year. In this study, 839 patients included between 1993 and 1999 were enrolled.

ESPOIR

The Evaluation et Suivi de POlyarthrites Indifférenciées Récentes (ESPOIR) is a cohort in which patients from 14 regional centers across France (16 university hospital rheumatology departments) were recruited.[26] Patients were aged 18-70 years and had ≥2 swollen joints for >6 weeks and <6 months and a high clinical suspicion on RA based on expert assessment. In total, 632 RA patients included between 2002 and 2005 were studied here.

Umeå

Umeå is an observational inception cohort in which 459 RA patients with symptom duration <12 months from four different counties in Sweden were included between 1995 and 2010.[12] The age ranged between 18 and 83 years.

Lund

This cohort study recruited patients from primary care units in the area of Lund, Sweden, and included patients with RA for <24 months aged 18-78 years.[27] Although at inclusion RA was defined using the 1958 criteria, these patients also fulfilled the 1987 criteria.[28-30] In total, 183 patients were included between 1985 and 1989; of these, 147 were previously evaluated in longitudinal studies[31,32] and also studied here.

Serological measurements

Baseline serum samples were tested for ACPA: Leiden EAC, anti-CCP2 Immunoscan RA Mark 2 (Eurodiagnostica, Arnhem), cut-off 25 U/ml, and anti-CCP2 EliA CCP (Phadia, Nieuwegein, the Netherlands), cut-off 7 U/ml, were used to determine the presence of ACPA, ACPA level was studied in samples tested with the anti-CCP2-test from Eurodiagnostica; ESPOIR, anti-CCP2 (DiaSorin, France), cut-off 50 U/ml; BARFOT and Umeå, anti-CCP2 (Eurodiagnostica, Malmö, Sweden), cut-off 25 U/ml; and Lund, anti-CCP2 (Anamar Lund, using commercial kits, Inova Diagnostics, San Diego, CA), cut-off 20 U/ml. IgM RF was determined as follows: Leiden EAC, in-house ELISA; BARFOT, Serodia RA agglutination test (Fujirebio Inc., Tokyo, Japan); ESPOIR: Elisa, Menarini, France; positive >9 UI/ml); Umeå, in-house ELISA; and Lund (ELISA, Anamar Lund, using commercial kits, Inova Diagnostics, San Diego, CA). IgG anti-CarP antibodies against carbamylated fetal calf serum were determined as described previously in the Leiden EAC,[33] cut-off for positivity was based on the mean +2 SD from a set of 204 healthy controls; and in BARFOT the cut-off was based on the 82 controls from the source population.[34]

ACPA characteristics

Data on ACPA isotypes, ACPA fine specificity and ACPA avidity were determined as described previously[35-37] in 157 RA patients included in the Leiden EAC. In short, by measuring ACPA isotypes different antibody subclasses can be distinguished which all differ in their ability to mediate effector responses.[38] ACPA IgG1, IgG2, IgG3, IgG4, IgA and IgM were determined using a sandwich ELISA.[37] The total number of ACPA isotypes in each ACPA-positive patient was used in our analysis. ACPA fine specificity was studied to measure an increase or shift in antigen recognition. To determine ACPA fine specificity, antibodies against the citrullinated and the arginine-containing form of two peptides derived from vimentin (Vim1-16; Vim59-74), two peptides derived from fibrinogen (Fibα 27-43; Fibβ 36-52) and one peptide derived from α-enolase (Eno 5-20) and against citrullinated myelin basic protein were determined by in-house ELISA.[36] The sum of citrullinated antigens recognized by ACPA in each patient was used in our analysis. Finally the avidity of ACPA IgG, as a measure of the strength of the ACPA response, was determined with elution ELISAs.[35] Avidity is presented as the relative avidity index, which was defined as the ratio of the amount of residual antibodies bound to the antigen-coated plate after NaSCN (1 M) elution to the amount of bound antibodies in the absence of NaSCN, expressed as a percentage.

Statistical analysis

To visually inspect the relation between age of onset and presence of autoantibodies, the proportion of autoantibody-positive and autoantibody-negative patients was plotted for different age groups of each 5 years. If <10 patients were present in the older age groups (BARFOT, Lund), age groups were summed. To statistically evaluate associations with age of onset, logistic regression analyses were performed per cohort with ACPA, RF or anti-CarP antibodies as the dependent variable and age of onset as the independent variable. Because descriptive results (Figure 8.1, Supplementary figure 8.1, 8.2, 8.3) showed that the proportion of autoantibody-positive RA patients decreased after ±50 years of age, a two-phase logistic regression analysis with one change point at 50 years was fitted. Odds ratios of the different cohorts (obtained from regression analyses) were entered in an inverse-weighted meta-analysis. This method weights results with a low standard error stronger than results with a higher standard error, thereby preventing over-representation of less precise data. A random effect model was used. The meta-analysis was performed separately for age of onset <50.0 years and >50.0 years and separately in males and females.

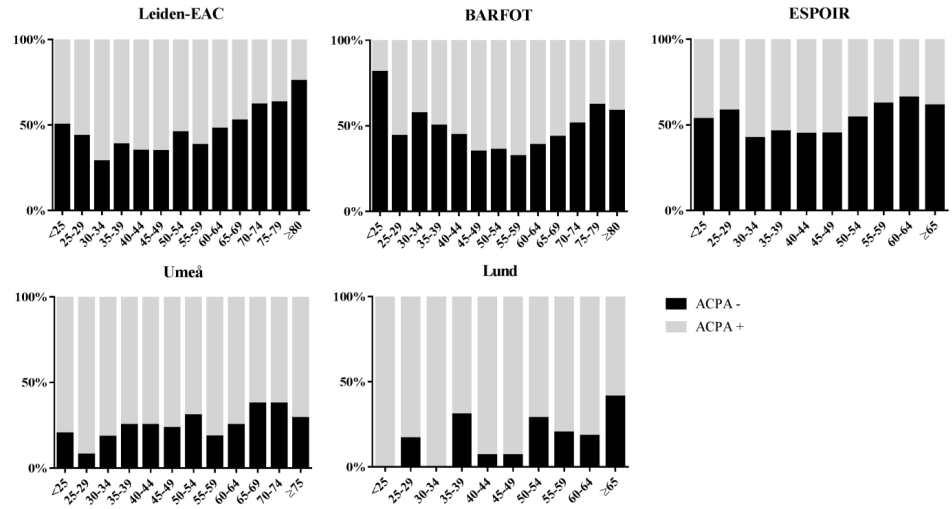

Figure 8.1 Proportion of ACPA-negative RA patients at different ages of RA onset within the different age groups at RA onset in the five different cohorts

Number of patients in each age group: Leiden EAC: <25, n=48; 25-29, n=23; 30-34, n=49; 35-39, n=65; 40-44, n=86; 45-49, n=121; 50-54, n=125; 55-59, n=144; 60-64, n=153; 65-69, n=122; 70-74, n=131; 75-79, n=92; ≥80, n=49; BARFOT: <25, n=16; 25-29, n=25; 30-34, n=42; 35-39, n=40; 40-44, n=45; 45-49, n=66; 50-54, n=92; 55-59, n=90; 60-64, n=75; 65-69, n=92; 70-74, n=82; 75-79, n=66; ≥80, n=29; ESPOIR: <25, n=30; 25-29, n=24; 30-34, n=45; 35-39, n=52; 40-44, n=65; 45-49, n=78; 50-54, n=107; 55-59, n=109; 60-64, n=73; ≥65, n=49; Umeå: <25, n=20; 25-29, n=13; 30-34, n=22; 35-39, n=28; 40-44, n=28; 45-49, n=43; 50-54, n=62; 55-59, n=60; 60-64, n=72; 65-69, n=48; 70-74, n=32; ≥75, n=31; Lund: <25, n=2; 25-29, n=6; 30-34, n=2; 35-39, n=13; 40-44, n=15; 45-49, n=30; 50-54, n=21; 55-59, n=25; 60-64, n=11; ≥65, n=17. ACPA, anti-citrullinated protein antibodies.

The proportion of ACPA-positive and ACPA-negative RA patients within the Leiden EAC was then compared with ACPA probabilities from the general Dutch population.[7] Within different age categories the risk of being ACPA-positive within the Leiden EAC was divided by the risk of being ACPA-positive within the general Dutch source population, revealing a risk ratio. The same was done for the risk of being ACPA-negative. Risk ratios of ACPA positivity and ACPA negativity were plotted for different age categories.

Data on ACPA characteristics were depicted visually for different age groups of each 10 years, and evaluated statistically with linear regression analysis (ACPA level, ACPA avidity) and ordinal regression analysis (ACPA isotypes, ACPA fine specificity), using Bonferroni correction for multiple testing.

Within the Leiden EAC, the association between age of onset and smoking, SE alleles and symptom onset was analyzed with logistic regression analysis, and with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and swollen joint count (SJC) with Spearman's correlation coefficient, using Bonferroni correction for multiple testing. Symptom onset was considered sub(acute) when there was prompt onset (e.g., <1 week), and not a gradual or intermittent onset. All regression analyses were adjusted for gender. Analyses were performed using SPSS version 23.0 (IBM).

Results

Patient characteristics

Baseline characteristics of all included patients are presented in Table 8.1. The majority of the included patients were female and the mean age of onset in the different cohorts ranged from 48.9 to 56.7 years. Symptom duration ranged from 18.3 to 43.3 weeks with the longest symptom duration observed in Lund. Within Leiden EAC, BARFOT and ESPOIR about 50% of the included patients were ACPA-positive, while in Umeå and Lund the percentage of ACPA-positive patients was 73.9% and 80.3%.

Table 8.1 Baseline characteristics of patients with rheumatoid arthritis included in the cohorts studied

variable	Leiden-EAC	BARFOT	ESPOIR	Umeå	Lund
Total number of patients	1244	839	632	459	147
Age, mean(sd)	56.6 (15.5)	56.7 (15.4)	48.9 (12.2)	53.9 (14.5)	50.7 (11.5)
Female, n(%)	827 (66.5)	538 (64.1)	484 (76.6)	321 (69.9)	98 (66.7)
Symptom duration[a], median (IQR), weeks	18.3 (9-36)	26.1 (17-39)	21.4 (13-33)	28.0 (16-39)	43.3 (28-61)
Smoking at baseline, n(%)	308 (25.9)	227 (27.1)	137 (21.7)	107 (23.9)	39 (30.7)
ACPA+, n(%)	638 (52.8)	418 (55.0)	291 (46.0)	339 (73.9)	114 (80.3)
RF+, n(%)	715 (58.0)	453 (59.6)	344 (54.4)	362 (79.0)	115 (81.0)
anti-CarP+, n(%)	474 (42.3)	280 (34.7)	NA	NA	NA
ESR (mm/h), median (IQR)	31 (16-50)	30 (15-50)	23 (12-41)	22 (12-39)	28 (13-50)
CRP (mg/L), median (IQR)	14 (6-35.5)	19 (7-47.5)	10 (3-26)	10 (8-25)	15 (0-45.5)
SJC, median (IQR)	5 (3-10)	10 (6-14)	7 (4-11)	6 (3-10)	6 (3-10)
TJC, median (IQR)	6 (2-11)	7 (3-12)	8 (4-14)	5 (2-10)	7 (4-11)

[a]Time between symptom onset and inclusion in cohort.
ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; n, number of patients; NA, not available; RF, rheumatoid factor; SD, standard deviation; SJC, swollen joint count based on 66 joints (Leiden EAC) or on 28 joints (BARFOT, ESPOIR, Umeå) or on 50 joints (Lund); TJC, tender joint count based on 68 joints (Leiden EAC) or on 28 joints (BARFOT, ESPOIR, Umeå) or Ritchie index (Lund).

ACPA prevalence decreased in RA patients with an older age at onset

The proportion of ACPA-positive RA was plotted for all age categories in all five cohorts (Figure 8.1). This showed that the proportion of ACPA-positive patients seemed to decrease after age of onset of 50 years. Logistic regression analyses with a change point at 50 years of age and with adjustment for gender were performed for each cohort; odds ratios (ORs) were combined in a meta-analysis. There was no association between the age of onset and the presence of ACPA in RA patients with an age of onset <50 years (OR 1.01, 95% CI 0.99-1.04). However, age of onset >50 years was associated with a lower frequency of ACPA positivity (OR 0.96, 95% CI 0.95–0.97; Figure 8.2A). An OR of 0.96 indicates that for a 1-year increase in the age of onset, the odds of being ACPA-positive decrease 4%; thus this reflects 18% per 5-year increase in age. Results were similar when studying ACPA in age categories of 5 years instead of continuously (Supplementary figure 8.1). Similar results were observed for RF and anti-CarP antibodies (Supplementary figure 8.2, Supplementary figure 8.3, Figure 8.2B, C).

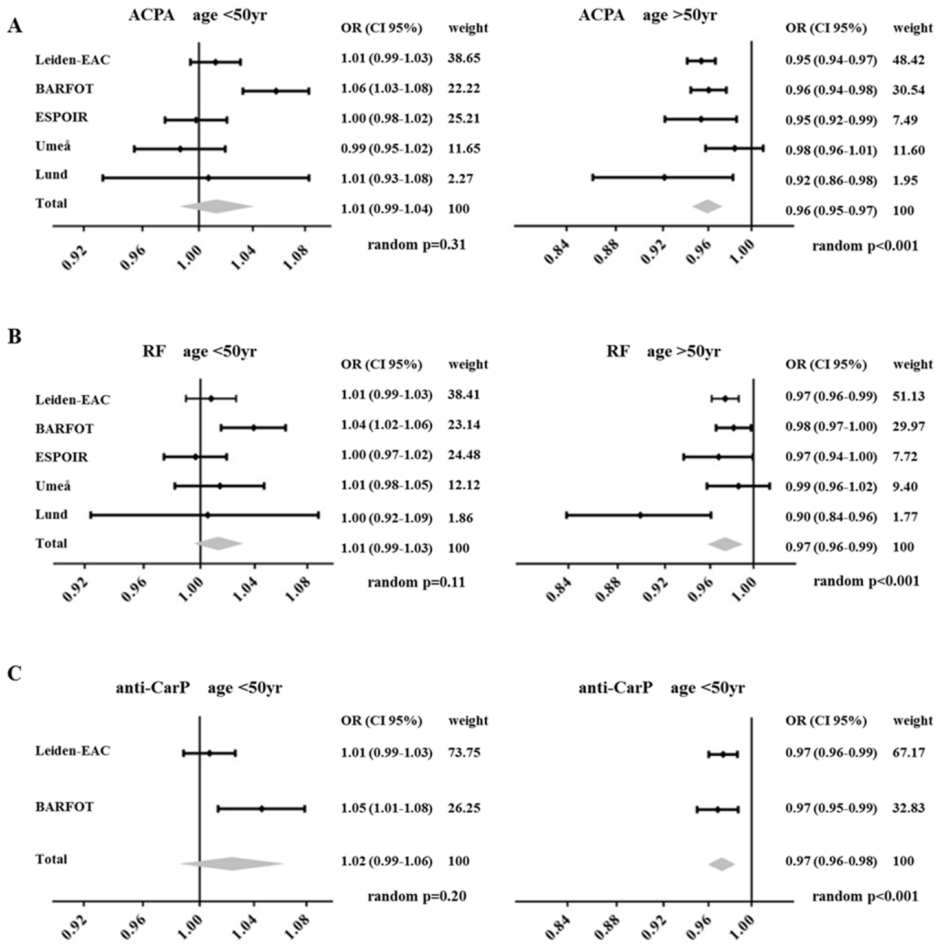

Figure 8.2 Meta-analysis on the association between age of onset (<50 and >50 years) and the presence of ACPA, RF and anti-CarP antibodies

Association between ACPA (A), RF (B) and anti-CarP antibodies (C) with age of onset in the different cohorts. The meta-analysis summarizes the effect of age of onset in the different cohorts and is based on a random effect model, combining ORs from separate logistic regression analyses of the different cohorts with age and gender as independent variables and ACPA, RF or anti-CarP antibodies as outcome. Separate meta-analyses were performed for the association between autoantibodies and age <50 years and >50 years. OR of 0.96 indicates that for a 1-year increase in age, the odds of being ACPA-positive decrease 4%; this is 18% per 5-year increase in age of RA onset (0.96^5). ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; OR, odds ratio; RF, rheumatoid factor.

Also when analyses were repeated with a change point at 60 years of age, similar results were obtained (meta-analysis: p<0.001 for an association between ACPA presence and age of onset in patients aged >60 years; and no significant association in patients aged <60 years, p=0.88).

Then we studied the proportion of ACPA-positive and ACPA-negative patients in relation to the ACPA prevalence of the Dutch source population (Figure 8.3). This showed that, for example, in the age group 18-29 the risk of being ACPA-positive was 87 times higher for RA patients compared with individuals from the general population. In line with this, the risk of being ACPA-negative was 0.48 times higher (meaning 52% lower) for RA patients compared with individuals from the general population. The risk ratio for ACPA-negativity increased at older age.

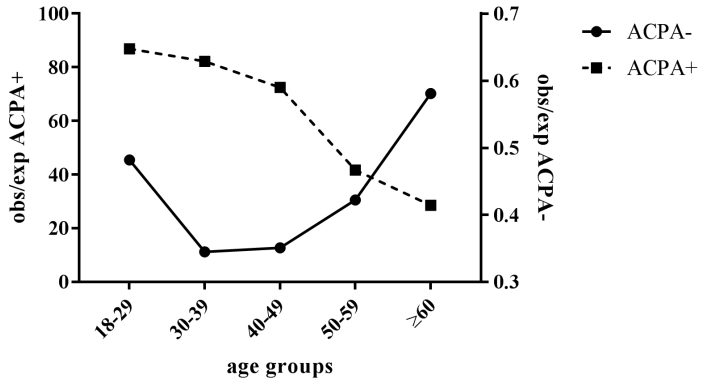

Figure 8.3 Risk of ACPA positivity and ACPA negativity in RA patients compared with individuals from the Dutch source population, presented for different age categories

For example, in the age group 18–29 the risk of being ACPA-positive was 87 times higher for RA patients than for individuals from the general Dutch population, and the risk of being ACPA-negative was 0.48 times higher (meaning 52% lower). The ratio for ACPA negativity increased at older age. ACPA, anti-citrullinated protein antibodies; obs, observed; exp, expected.

ACPA characteristics did not differ for different ages of RA onset

After having studied the presence of ACPA-positive RA, several characteristics of the ACPA response were evaluated within ACPA-positive RA patients. First, ACPA level was analyzed in relation to age of onset; no association between ACPA level and age of onset was observed (Leiden EAC p=0.49, BARFOT p=0.21, ESPOIR p=0.91, Umeå p=0.34, Lund p=0.08; Figure 8.4). Then within the Leiden EAC the number of ACPA isotypes was evaluated because isotype class switching can lead to an increased diversity of the antibody repertoire. The ordinal regression showed p=0.03, and was not significant after correcting for multiple testing (cut-off Bonferroni correction p=0.01, Figure 8.5A). No association was observed between age at onset and the ACPA fine specificity (which we presented as the number of recognized citrullinated antigens by ACPA, p=0.96; Figure 8.5B) and the ACPA

avidity index (which measures the overall binding strength of the ACPA response to CCP2, p=0.62; Figure 8.5C). These findings together suggest that the analyzed ACPA characteristics were comparable within different age categories.

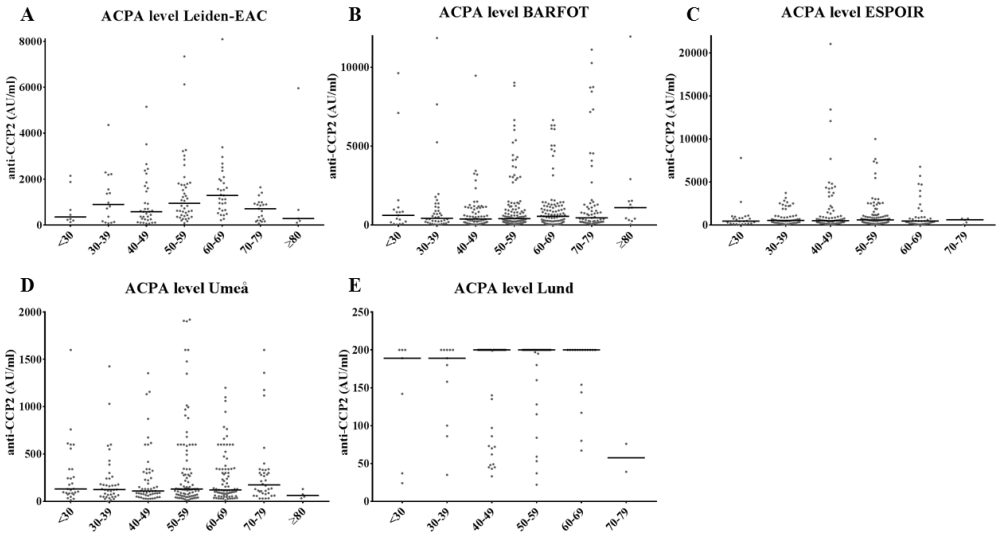

Figure 8.4 ACPA level of ACPA-positive RA at different ages of RA onset; data from five cohorts

Association between age of onset and ACPA level within RA patients of the Leiden EAC (A), BARFOT (B), ESPOIR (C), Umeå (D) and Lund (E) cohorts. In Lund the upper detection limit of the anti-CCP2 test was 200 U/ml; there were 76 patients with anti-CCP2 level >200 U/ml. Horizontal lines represent median values. Each dot represents one patient. ACPA, anti-citrullinated protein antibodies; anti-CCP2, anti-cyclic citrullinated peptide 2.

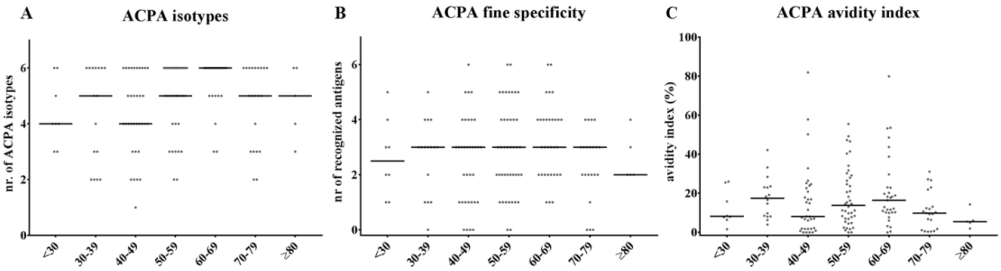

Figure 8.5 Isotypes, fine specificity and avidity index of ACPA-positive RA patients at different ages of onset; data from the Leiden EAC

Association between age of onset and ACPA isotypes (A), ACPA fine specificity (B) and ACPA avidity index (C) within RA patients of the Leiden EAC. Horizontal lines represent median values. Each dot represents one patient. ACPA, anti-citrullinated protein antibodies.

Several clinical parameters in RA patients at disease onset associated with age of onset

The decrease in the relative proportion of ACPA-positive RA patients with increasing age of onset was not paralleled by age-related differences in the ACPA response itself, which suggests that an age-dependent effect on the ACPA response was not the most likely explanation. To further substantiate this, the associations of age with smoking and the HLA-SE alleles were determined, because these are the main risk factors for ACPA-positive RA. The presence of SE alleles remained constant over age of onset ($p=0.54$), but the proportion of smokers decreased with increasing age ($p<0.001$, Supplementary figure 8.4). Similar to that observed for ACPA, this decrease was most prominent for RA patients with an age of onset >50 years.

Another explanation for the higher proportion of ACPA-negative RA at older age of onset is that a group of (autoantibody-negative) patients with different etiopathology was preferentially present at older age and classified as RA. Because some previous studies have reported associations between clinical characteristics (male gender, more often acute onset, higher acute phase reactants) and an older age of onset,[4-6] we aimed to substantiate this in the present data. We evaluated whether the association between age and the presence of autoantibodies was similar in males and females, showing that the effect was more pronounced in males (Supplementary figure 8.5). Furthermore, an older age of onset was associated with higher CRP levels ($\rho=0.26$, $p<0.001$), higher ESR levels ($\rho=0.32$, $p<0.001$) and a higher number of swollen joints ($\rho=0.10$, $p=0.001$) at first presentation. RA patients presenting at older age also more often had (sub)acute onset of symptoms ($p=0.003$, Supplementary figure 8.6). These findings remained significant after Bonferroni correction (cut-off $p=0.008$).

Altogether these data suggest that at older age there is a subgroup of patients who fulfil the classification criteria for RA that is more often male, nonsmoking, has higher acute phase reactants, more often has (sub)acute symptom onset and is also more often ACPA-negative.

Discussion

Previous studies have evaluated differences in relation to the age of onset of RA, and have shown that some clinical characteristics were more prevalent at an older age of onset. Whether or not the ratio of ACPA-positive and ACPA-negative

RA was also different was unresolved until now because different studies have yielded contrasting results. This prompted us to perform the present study in 3321 RA patients from five RA cohorts. The combination of the present data clearly showed that the proportion of autoantibody-positive patients (i.e., ACPA, RF and anti-CarP antibodies) was lower in RA patients who presented at older age. We also studied characteristics of the ACPA response, and within ACPA-positive RA patients characteristics of this response did not appear to differ at different ages of onset. Hence, our results suggest that the composition of the ACPA response is not different, but only the proportion of ACPA-positive RA is lower at older age of onset. In other words, the data revealed that ACPA-negative RA was more prevalent at older age.

Some findings within RA patients are different from findings obtained in the general population. In the general population, ageing is associated with lower antibody levels in response to vaccination.[21] In this study there was no association between ACPA level and age of onset. In addition, in the population autoantibodies (such as antinuclear antibodies, RF and ACPA) are increasingly prevalent at older age,[7,39-41] whereas within RA patents we observed a higher proportion of ACPA-negative disease at older age. This difference also resulted in the observation made in Figure 8.3.

Interestingly, not only the proportion of ACPA-positive RA decreased with an older age at onset but also the proportion of RA patients who smoked at disease onset. This observation corresponds to the prevalence of present smokers in the general population, which decreases around 50 years of age.[42] Smoking is a known risk factor for ACPA-positive RA[43] and it is intriguing to speculate that a decrease in smoking patients (compared with nonsmokers) mediates the lower proportion of ACPA-positive RA at older age.

The 2010 classification criteria for RA could not be used to classify RA in the present study because of circularity between the dependent and independent variables. According to the 1987 criteria, RA is mainly classified based on clinical features, among which is symmetric polyarthritis of small joints. Our data suggest that patients fulfilling the 1987 criteria at older age more often had slight differences in other baseline characteristics, because they were more often males, had higher acute phase reactants and more often had (sub)acute onset of symptoms. Cluster analysis using only clinical characteristics, however, was insufficient to cluster patients on the individual level (data not shown). Nonetheless, based on the present data we presume that part of the ACPA-negative RA patients presenting at older age constitute a subgroup with slight differences in clinical presentation

but with more pronounced differences in underlying pathogenic mechanisms. Biologic studies are now warranted to further evaluate this hypothesis and to identify a distinct subgroup within the seronegative patients.

A potential limitation is that the five cohorts were not completely comparable and that two cohorts contained an overall higher percentage of ACPA-positive patients than the other cohorts. Probably this can be explained by differences in health care systems or settings. When for instance the presence of ACPA (or other characteristics of more severe disease) is considered more relevant in the referral process or for inclusion in cohorts, this could explain the higher percentage of ACPA-positive patients in these cohorts. Nonetheless, here the percentage of ACPA-negative patients was also higher at older age of onset. The measurement of ACPA was not centralized, which may have led to different misclassification in different cohorts. Furthermore, anti-CarP antibodies were determined in only two of the five cohorts. We observed that RF and anti-CarP antibodies also decreased with increasing age of onset, although less distinctly than ACPA. The different autoantibodies often occur in the same patients; therefore another limitation is that we have not studied whether the decrease of RF and anti-CarP antibodies was independent of the age-related decrease of ACPA.

A final limitation is that studies on ACPA fine specificity, ACPA isotypes and ACPA avidity index were less powered than those on ACPA level. However, it is known that ACPA level is highly associated with ACPA fine specificity and the number of ACPA isotypes.[44] Because ACPA level was determined in all cohorts and there was no tendency toward differences in ACPA level in patients aged >50 years at RA onset, this may suggest that ACPA fine specificity and ACPA isotypes would also remain stable with increasing age of RA onset. In some cohorts, patients aged >80 appeared to have lower ACPA levels, although this age group contained very few patients.

Conclusions

Characteristics of the ACPA response in ACPA-positive RA patients did not appear to be age dependent, while data of five cohorts revealed that with older age of onset ACPA-negative RA is more frequent than ACPA-positive RA. Further biologic studies are needed to characterize the pathogenesis of ACPA-negative polyarthritis at older age and to promote personalized treatment decisions in ACPA-negative patients in daily practice.

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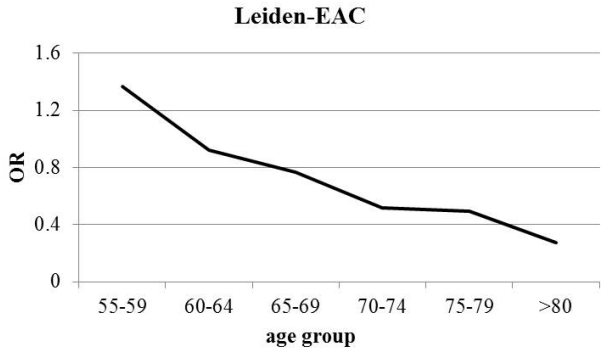
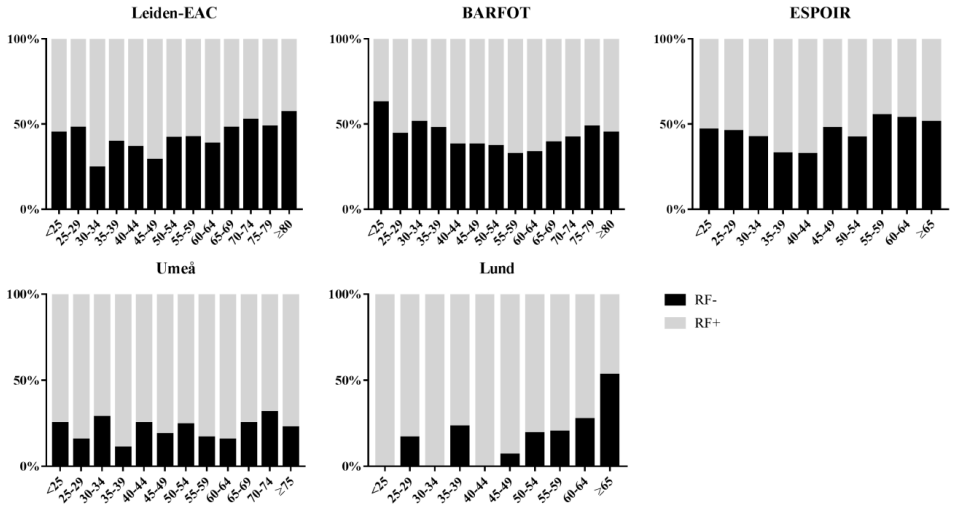
Supplementary material



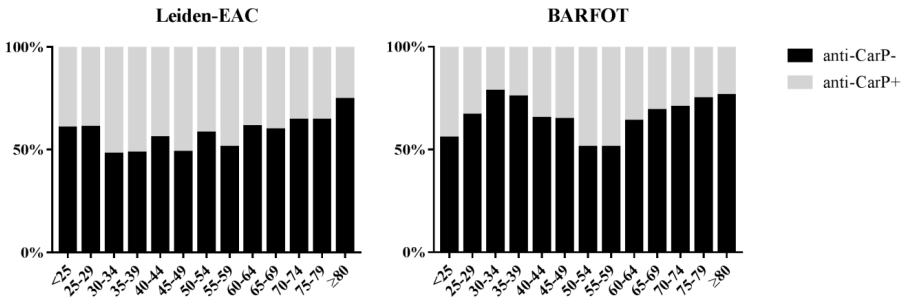
Supplementary figure 8.1 Association between age and ACPA within the Leiden-EAC with age in categories of 5 years

Logistic regression analyses were performed with the presence of ACPA as outcome variable and gender and age as independent variables. Age was studied as a categorical variable (age groups of 5 years), with the age group 50-54 as the reference group. The ORs for ACPA positivity decreased linearly with increasing age groups. OR, odds ratio.



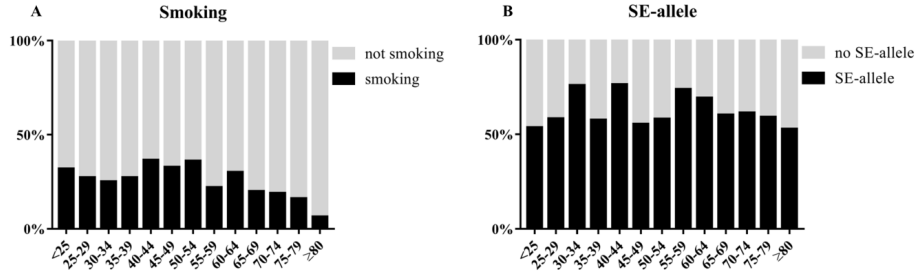
Supplementary figure 8.2 Proportion of RF-negative RA patients at different ages of RA onset; data from five cohorts

Presented are the proportion RF-negative and RF-positive RA patients within the different age groups in the five different cohorts. Number of patients in each age group: Leiden EAC: <25, n=49; 25-29, n=23; 30-34, n=49; 35-39, n=66; 40-44, n=88; 45-49, n=125; 50-54, n=127; 55-59, n=147; 60-64, n=156; 65-69, n=128; 70-74, n=130; 75-79, n=93; ≥80, n=51; BARFOT: <25, n=16; 25-29, n=25; 30-34, n=43; 35-39, n=40; 40-44, n=45; 45-49, n=66; 50-54, n=92; 55-59, n=90; 60-64, n=75; 65-69, n=92; 70-74, n=81; 75-79, n=66; ≥80, n=29; ESPOIR: <25, n=30; 25-29, n=24; 30-34, n=45; 35-39, n=52; 40-44, n=65; 45-49, n=78; 50-54, n=107; 55-59, n=109; 60-64, n=73; ≥65, n=49; Umeå: <25, n=20; 25-29, n=13; 30-34, n=21; 35-39, n=28; 40-44, n=28; 45-49, n=43; 50-54, n=62; 55-59, n=60; 60-64, n=72; 65-69, n=48; 70-74, n=32; ≥75, n=31; Lund: <25, n=2; 25-29, n=6; 30-34, n=2; 35-39, n=13; 40-44, n=15; 45-49, n=30; 50-54, n=21; 55-59, n=25; 60-64, n=11; ≥65, n=17. RA, rheumatoid arthritis; RF, rheumatoid factor.



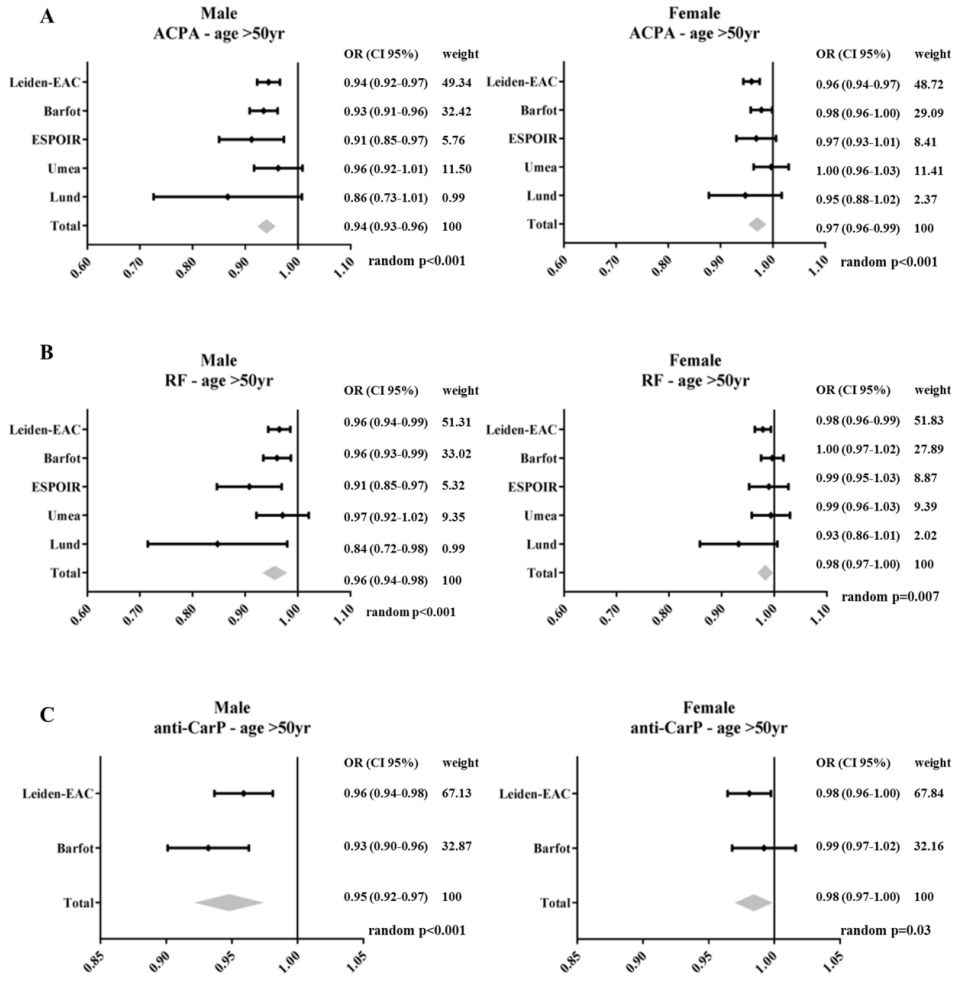
Supplementary figure 8.3 Proportion of anti-CarP-negative RA patients at different ages of RA onset; data from two cohorts

Presented are the proportion of anti-CarP-negative and anti-CarP-positive RA patients within the different age groups in the Leiden EAC and BARFOT cohorts. Number of patients in each age group: Leiden EAC: <25, n=43; 25-29, n=23; 30-34, n=44; 35-39, n=62; 40-44, n=79; 45-49, n=111; 50-54, n=112; 55-59, n=135; 60-64, n=144; 65-69, n=114; 70-74, n=120; 75-79, n=87; ≥80, n=47; BARFOT: <25, n=18; 25-29, n=27; 30-34, n=37; 35-39, n=41; 40-44, n=43; 45-49, n=62; 50-54, n=96; 55-59, n=88; 60-64, n=80; 65-69, n=103; 70-74, n=88; 75-79, n=87; ≥80, n=38. RA, rheumatoid arthritis; anti-CarP, anti-carbamylated protein antibodies.



Supplementary figure 8.4 Proportion of present smokers and presence of SE alleles at different ages of onset of RA; data from the Leiden EAC

Presented are the proportion of currently smoking RA patients (n=308) versus not smoking (none and past smoking) RA patients (n=880) (A) and the proportion of patients carrying one or two SE alleles (n=467) versus no SE alleles (n=272) (B) within different age groups in the Leiden EAC. Number of patients in each group: smoking: <25, n=47; 25-29, n=22; 30-34, n=48; 35-39, n=66; 40-44, n=85; 45-49, n=119; 50-54, n=125; 55-59, n=141; 60-64, n=153; 65-69, n=121; 70-74, n=127; 75-79, n=87; ≥80, n=47; SE alleles: <25, n=28; 25-29, n=12; 30-34, n=29; 35-39, n=40; 40-44, n=59; 45-49, n=74; 50-54, n=81; 55-59, n=80; 60-64, n=91; 65-69, n=73; 70-74, n=75; 75-79, n=61; ≥80, n=36. RA, rheumatoid arthritis; SE, shared epitope.



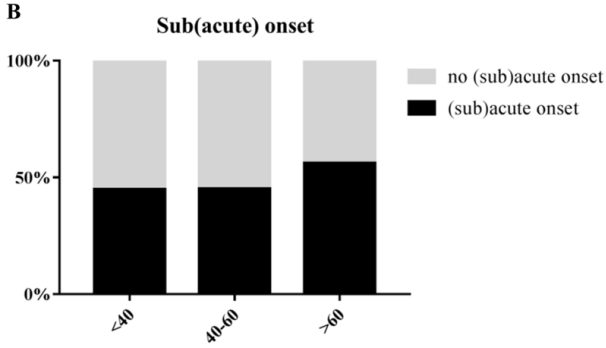
Supplementary figure 8.5 Meta-analysis on the association between age of onset and the presence of ACPA, RF and anti-CarP antibodies in male and female RA patients

Association between ACPA (A), RF (B) and anti-CarP antibodies (C) with age of onset in the different cohorts for males and females separately. The meta-analysis summarizes the effect of age of onset in the different cohorts and is based on a random effect model, combining the ORs from separate logistic regression analyses of the different cohorts with age as the independent variable and ACPA, RF or anti-CarP antibodies as outcome. Only the meta-analyses on the association between autoantibodies and age >50 years at RA diagnosis are shown. OR of 0.94 indicates that for a 1-year increase in age of onset, the odds of being ACPA-positive decrease 6%; this is 27% per 5-year increase in age of onset (0.945). ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; OR, odds ratio; RA, rheumatoid arthritis; RF, rheumatoid factor.

A

Outcome: (sub)acute onset			
	OR	95% CI	p-value
constant	0.44		0.002
age at RA-onset, years	1.01	1.00-1.02	0.003
male sex	1.15	0.90-1.47	0.252

B



Supplementary figure 8 .6 Association between age of onset and onset of symptoms within RA patients of the Leiden EAC.

(A) Results of logistic regression analyses of age at RA onset in relation to the onset of symptoms. OR of 1.01 indicates that per 1-year increase in the age of onset, the odds of having (sub)acute onset increase 1%. This reflects 12% (1.01^{10}) per 10-year increase in age of onset and 25% (1.01^{20}) per 20-year increase in age of onset. (B) Proportion of RA patients with (sub)acute onset of symptoms in three age groups (p=0.003). Number of patients per age group: <40, n=181; 40-60, n=466; >60, n=537. CI, confidence interval; OR, odds ratio; RA, rheumatoid arthritis.

PART III

Resolution of rheumatoid arthritis

9

Does immunological remission, defined as disappearance of autoantibodies, occur with current treatment strategies? A long-term follow-up study in rheumatoid arthritis patients who achieved sustained DMARD-free status

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Abstract

Objectives

Sustained disease-modifying antirheumatic drug (DMARD)-free status, the sustained absence of synovitis after cessation of DMARD therapy, is infrequent in autoantibody-positive rheumatoid arthritis (RA), but approximates cure (i.e., disappearance of signs and symptoms). It was recently suggested that immunological remission, defined as disappearance of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF), underlies this outcome. Therefore, this long-term observational study determined if autoantibodies disappear in RA patients who achieved sustained DMARD-free remission.

Methods

We studied 95 ACPA-positive and/or RF-positive RA patients who achieved DMARD-free remission after median 4.8 years and kept this status for the remaining follow-up (median 4.2 years). Additionally, 21 autoantibody-positive RA patients with a late flare, defined as recurrence of clinical synovitis after a DMARD-free status of ≥1 year, and 45 autoantibody-positive RA patients who were unable to stop DMARD therapy (during median 10 years) were studied. Anti-cyclic citrullinated peptide 2 (anti-CCP2) IgG, IgM and RF IgM levels were measured in 587 samples obtained at diagnosis, before and after achieving DMARD-free remission.

Results

13% of anti-CCP2 IgG-positive RA patients had seroreverted when achieving remission. In RA patients with a flare and persistent disease this was 8% and 6%, respectively (p=0.63). For anti-CCP2 IgM and RF IgM, similar results were observed. Evaluating the estimated slope of serially measured levels revealed that RF levels decreased more in patients with than without remission (p<0.001); the course of anti-CCP2 levels was not different (p=0.66).

Conclusions

Sustained DMARD-free status in autoantibody-positive RA was not paralleled by an increased frequency of reversion to autoantibody negativity. This form of immunological remission may therefore not be a treatment target in patients with classified RA.

Introduction

Sustained disease-modifying antirheumatic drug (DMARD)-free status is defined as sustained absence of synovitis after cessation of all DMARD therapy and is increasingly achievable by patients with rheumatoid arthritis (RA).[1] This status is also characterised by normalisation of functional status and lower levels of fatigue, pain and morning stiffness and is currently considered the best possible outcome of RA.[1] Absence of anti-citrullinated protein antibodies (ACPA) at disease presentation is an important predictor of achievement of sustained DMARD-free remission; however, with current treatment strategies this outcome is also observed in 10% of ACPA-positive RA.[1-5]

The pathophysiological role of ACPA in RA development or progression is not exactly known. ACPA can be present years before the onset of joint symptoms and disease, indicating that the mere presence of ACPA is not enough to develop disease.[6,7] Studies in the preclinical phase have shown that the ACPA immune response matures once disease onset is approached, as characterised by an increase in ACPA level, isotype-usage, avidity and the number of citrullinated epitopes recognised by ACPA.[8-11] In addition, there are changes in Fc glycosylation before RA onset.[12] Once RA is established the ACPA immune response does not mature any further.[13] Besides ACPA, also rheumatoid factor (RF) can be present years before disease onset.[6,7] Since autoantibodies are considered to have a prominent role in seropositive RA and precede symptom development, it is tempting to hypothesise that changes in the autoantibody response occur before or at the time when clinical disease has been extinguished, as is the case when sustained DMARD-free remission is reached. In this light, it was recently suggested that disappearance of autoantibodies is a hallmark of immunological remission and might characterise patients who are able to achieve drug-free remission.[14]

However, so far this hypothesis has not been thoroughly investigated. In a few studies, seroconversion and seroreversion during follow-up of early arthritis and RA patients were investigated. The observations described indicate that both are infrequent and not associated with relevant outcomes such as radiographic damage, functional status or the disease activity score.[15-18] In only one study, the association between seroreversion and drug-free remission was analysed and no association was observed.[19] However, autoantibody levels were only determined at disease presentation and at 1 year of follow-up, thus generally years before achievement of drug-free remission. In addition, follow-up of patients after the

achievement of drug-free remission was limited.[19]

We aimed to increase the understanding of the long-term course of RA-related autoantibodies in patients who had achieved the closest available proxy of cure of RA. Therefore, we investigated the association between ACPA and RF seroreversion and achievement of sustained DMARD-free remission in a unique population of RA patients with available serum samples at the time of remission and with a long follow-up duration after achievement of DMARD-free status.

Methods

Patients

Patients were retrieved from the Leiden Early Arthritis Clinic cohort, which is an inception cohort that includes patients with clinically confirmed arthritis and symptom duration <2 years. At baseline, patients and rheumatologists completed questionnaires, joint counts were performed and blood samples were collected. Follow-up visits were scheduled and blood samples were taken at 3-4 months, 6-8 months, 12 months, 18 months, 24 months and yearly thereafter. Between 1993 and 2014, 3473 patients were consecutively included, of which 1586 patients had a clinical diagnosis of RA and also fulfilled 1987 or 2010 RA classification criteria during the first year of follow-up.[20,21] Of these, 941 patients were ACPA-positive and/or RF-positive at baseline.

Treatment strategies changed over time. In general, patients included in 1993-1995 were initially treated with non-steroidal anti-inflammatory drugs, patients included in 1996-1998 with mild DMARDs (hydroxychloroquine or sulphasalazine) and patients included ≥1999 were initially treated with methotrexate. When this treatment failed, another conventional DMARD was initiated or added. A biological DMARD was allowed in patients who failed on ≥2 conventional DMARDs. Medication used by all studied patients during the observed follow-up period is shown in Table 9.1. Disease activity score (DAS44)-guided treatment became common from 2005 onwards with tapering and eventually stopping of treatment if DAS44 remained <2.4 and synovitis was absent at clinical joint examination, and intensifying treatment in case of DAS44 ≥2.4.

Table 9.1 Medication use during the total follow-up period; stratified by patient group

	DMARD-free sustained remission (n=95)	Flare after DMARD-free remission (n=21)	Persistent RA (n=45)
Methotrexate, n(%)	76 (80)	21 (100)	44 (98)
Other conventional DMARDs, n(%)	46 (48)	11 (52)	37 (82)
Sulfasalazine, n(%)	27 (28)	6 (29)	28 (62)
Hydroxychloroquine, n(%)	26 (27)	8 (38)	32 (71)
Leflunomide, n(%)	9 (9)	5 (24)	17 (38)
Azathioprine, n(%)	1 (1)	0 (0)	3 (7)
Cyclosporine, n(%)	0 (0)	1 (5)	0 (0)
Gold, n(%)	2 (2)	0 (0)	1 (2)
Biological DMARD, n(%)	14 (15)	6 (29)	19 (42)
TNF-inhibitor, n(%)	11 (12)	4 (19)	17 (38)
Rituximab, n(%)	1 (1)	1 (5)	2 (4)
Abatacept, n(%)	0 (0)	0 (0)	3 (7)
Tocilizumab, n(%)	2 (2)	1 (5)	3 (7)
Omalizumab, n(%)	0 (0)	0 (0)	1 (2)

Numbers indicate the number of patients who used the indicated medication at any time during follow-up; therefore, the indicated percentages for the different groups do not add up to 100%. The duration that patients used the medication and the number of patients using combination therapy is not indicated here. DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; TNF, tumor necrosis factor.

Of the 1586 RA patients, medical files were studied on occurrence of sustained DMARD-free remission until April 2017. This outcome was defined as absence of synovitis (by physical examination) after cessation of all DMARD therapy (including biologics and systemic and intra-articular corticosteroids) for at least 1 year and for the remainder of follow-up. Patients who experienced a flare of clinical synovitis early or late after DMARD cessation were considered as not in sustained DMARD-free remission. The date of sustained DMARD-free remission was the date 1 year after DMARD cessation. Patients who did not achieve remission were censored at the date when medical files were explored or at an earlier date when they were lost to follow-up or had died. Ninety-five of 941 ACPA-positive and/or RF-positive RA patients achieved sustained DMARD-free remission after a median follow-up of 4.8 years (Figure 9.1). After achievement of sustained DMARD-free remission, patients were additionally followed for median 4.2 years. Except for 1 patient, all patients were included from 1999 onwards.

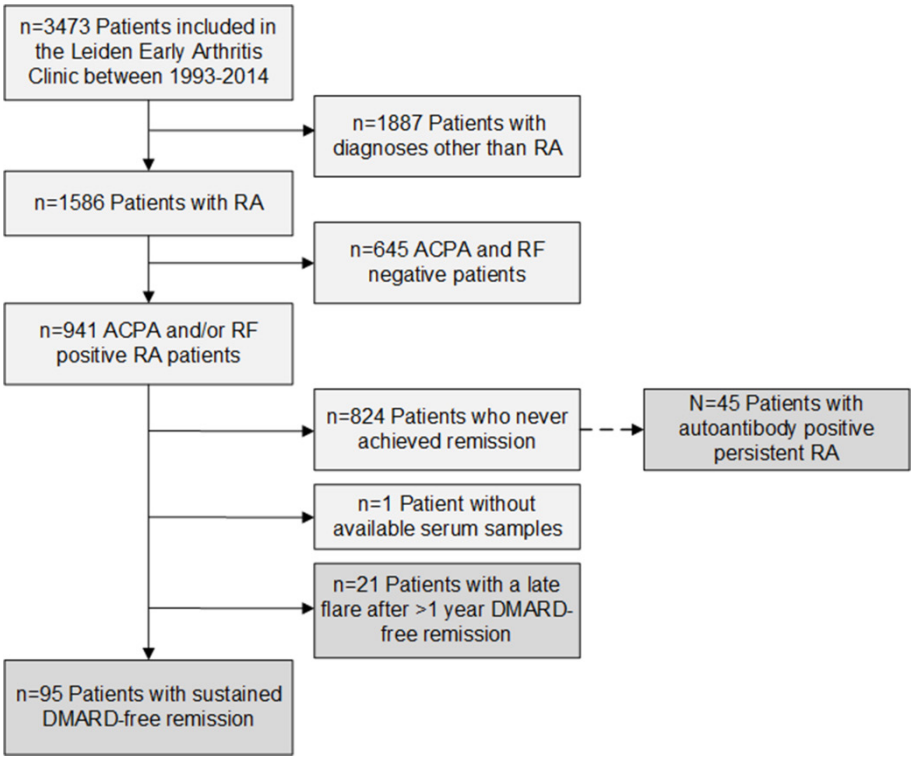

Figure 9.1 Flowchart of patient selection

DMARD-free remission was defined as the absence of clinical synovitis for ≥1 year after DMARD cessation. Flares were defined as recurrence of synovitis after having achieved DMARD-free remission, thus recurrence of synovitis >1 year after DMARD cessation. Sustained DMARD-free remission was defined as absence of synovitis after DMARD cessation during the complete follow-up, but at least for 1 year. As control, 45 autoantibody-positive patients with persistent RA were selected from the group of autoantibody-positive RA patients who never achieved DMARD-free remission based on comparable inclusion period as patients who achieved sustained remission and based on available serum samples. ACPA, anti-citrullinated protein antibodies; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

In addition, 21 autoantibody-positive RA patients who experienced a late flare were studied. These patients had absence of clinical synovitis for ≥1 year after DMARD cessation, thus were initially in DMARD-free remission. However, this remission was not sustained since these patients had recurrence of synovitis and needed to restart DMARDs during the remainder of follow-up. The median duration of being in DMARD-free remission before a flare occurred was 2.2 years. As control, 45 autoantibody-positive RA patients who were unable to stop DMARD therapy because of persistent swollen joints during follow-up, were evaluated. These patients were selected from the group of autoantibody-positive RA patients

who never achieved DMARD-free remission based on comparable inclusion period and on available serum samples at baseline, 1 year and at 7-8 years follow-up or earlier in case patients had a shorter follow-up duration.

Median total follow-up of the studied patients was 10 years and was comparable between the studied groups.

Serological measurements

Anti-cyclic citrullinated peptide 2 (anti-CCP2) IgG and RF IgM were measured in 587 serum samples obtained at diagnosis, before and after achieving DMARD-free remission, using enzyme-linked immunosorbent assays as described previously.[22,23] In all cases, <5% of controls were autoantibody-positive with the cut-offs used. In short, for IgM-RF, human IgG1 was used as the capture antigen and bound antibodies were detected with F(ab')2 fragments of peroxidase-conjugated antihuman IgM. The cut-off for positivity was 8 IU/mL. For anti-CCP2 IgG, the anti-CCP2 test (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands) was used with a cut-off value of 25 units/mL, as described in the manufacturer's instructions. An overview of the measured samples during follow-up for the different groups is depicted in Figure 9.2. Of each patient, a median of three samples was measured. In addition, anti-CCP2 IgM was measured in the first and last available serum sample of patients who were positive for anti-CCP2 IgG at disease presentation. In brief, microtiter plates were coated with citrullinated CCP2. An arginine control was used as control for citrulline specificity of the anti-CCP antibodies. Plates were incubated for 1 hour at 37°C with serum samples, 50 µL/well, at a dilution of 1:50. To detect anti-CCP2 IgM, plates were incubated for 1 hour at 37°C, 50 µL/well, with peroxidase-conjugated anti-human IgM. Pooled serum samples of highly positive patients were used in all plates to generate standard curves. Autoantibody levels were estimated by interpolation from these standard curves and were expressed in arbitrary units per millilitre. Samples were considered positive when the signal was higher than the mean +2 SD of serum samples of 64 healthy control subjects in total. This resulted in a cut-off value of 12 AU/mL. In addition, to ascertain that the obtained signal within the anti-CCP2 IgM ELISA was citrulline-specific, the difference between the signal against the citrullinated peptide and the unmodified arginine peptide had to be >0.1 (OD >0.1).

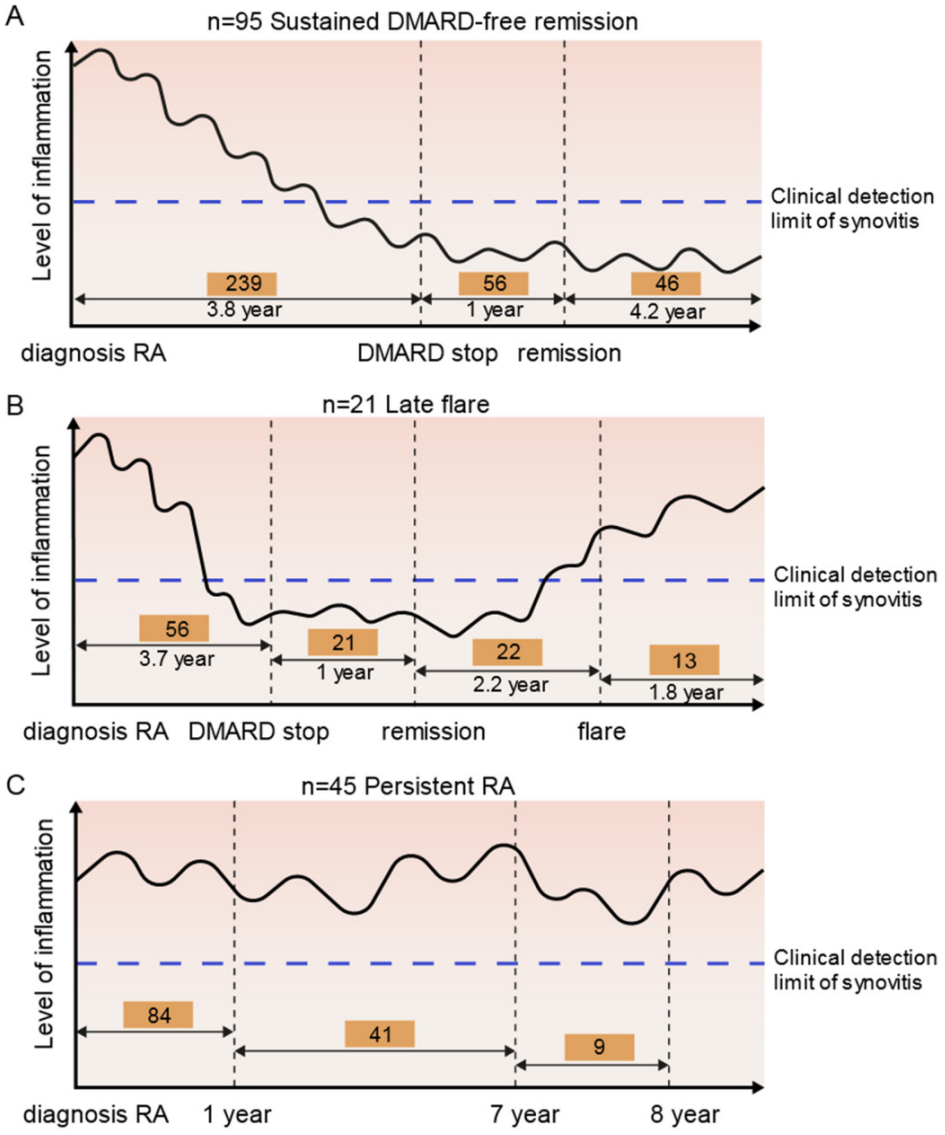

Figure 9.2 Overview of samples measured during follow-up of patients who achieved sustained DMARD-free remission (A), patients with a late flare (B) and patients with persistent RA (C)

Numbers indicate the number of samples measured within the indicated time periods. In total, 587 samples were measured. Of patients who achieved sustained DMARD-free remission, samples were obtained at diagnosis, before and at or after achieving remission. DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis.

Statistical analyses

The difference between the first and last available serum sample was used to calculate the proportion of patients with seroreversion. This was performed separately for patients positive for anti-CCP2 IgG, anti-CCP2 IgM and RF IgM. Differences in seroreversion between the three patient groups were compared with the Fisher exact test. To test whether changes in ACPA and/or RF levels during follow-up were associated with achievement of sustained DMARD-free remission, Cox proportional hazards regression analyses were performed with time till achievement of remission as outcome. For these analyses, patients with a late flare were combined with patients with persistent RA as one group. Changes in anti-CCP2 IgG and RF IgM level per year were estimated with linear regression analyses for each patient individually. These changes in levels over time were used as predictor in Cox proportional hazards regression analyses. Antibody levels below the detection limit were imputed with a value of 0.

Several subanalyses were performed to study whether results on seroreverion could be ascribed to variation around the cut-off level, whether results were different when groups were stratified for autoantibody combinations, whether results were different in patients who were treated early, or whether results were dependent on the follow-up duration. Finally, for RF a second cut-off for RF positivity was used. This was done as the cut-off that is used in clinical practice has a specificity of 95% when compared with healthy controls, but a reduced specificity when patients with other arthritides were used as reference.[24] A cut-off of 33 allowed a specificity of 98% relative to patients with other early arthritides in our cohort (data not shown) which was then equal to the specificity of ACPA.

SPSS V.23.0 was used and p-values <0.05 were considered significant.

Results

Patient characteristics

Baseline characteristics of the studied autoantibody-positive patients are presented in Table 9.2 and are similar between the different groups, with the exception that patients who achieved sustained DMARD-free remission were less frequently ACPA-positive.

Table 9.2 Baseline characteristics of RA patients who achieved sustained DMARD-free remission, who flared after being in DMARD-free remission and of patients with persistent RA

	Sustained DMARD-free remission (n=95)	Late flare after ≥1 year of DMARD-free remission (n=21)	Persistent RA (n=45)	p-value
Age in years, mean (SD)	54 (17)	52 (13)	55 (12)	0.63
Female, n (%)	65 (68)	13 (62)	30 (67)	0.85
Symptom duration in weeks, median (IQR)	17 (10-35)	20 (8-37)	20 (13-38)	0.56
66-SJC, median (IQR)	5 (3-9)	5 (2-13)	5 (3-8)	0.81
68-TJC, median (IQR)	8 (3-13)	12 (4-15)	5 (4-11)	0.32
Autoantibody status				0.047
anti-CCP2 IgG+ RF IgM-, n(%)	9 (9)	0 (0)	4 (9)	
anti-CCP2 IgG- RF IgM+, n(%)	41 (43)	6 (29)	10 (22)	
anti-CCP2 IgG+ RF IgM+, n(%)	45 (47)	15 (71)	31 (69)	
CRP (mg/L), median (IQR)	11 (3-28)	11 (4-23)	14 (5-40)	0.39
ESR (mm/h), median (IQR)	26 (14-49)	29 (13-44)	29 (20-41)	0.75

Patients with persistent RA were selected from the group of autoantibody-positive RA patients who never achieved DMARD-free remission based on comparable inclusion period as patients who achieved sustained remission and based on available serum samples. anti-CCP2, anti-cyclic citrullinated peptide 2; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; IQR, interquartile range; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; SJC, 66-swollen joint count; symptom duration, time between symptom onset and inclusion in cohort; TJC, 68-tender joint count.

Anti-CCP2 IgG and RF IgM seroreversion were not associated with achievement of sustained DMARD-free remission

First, anti-CCP2 IgG levels were serially measured in the three different patient groups (Figure 9.3A). Of anti-CCP2 IgG-positive RA patients who achieved sustained DMARD-free remission, 13% had reverted to anti-CCP2 IgG negativity around the time of remission (Figure 9.4A). However, for RA patients with a late flare or with persistent disease, seroreversion was observed in 8% and 6%, respectively, which was not significantly different from patients who achieved sustained DMARD-free remission (p=0.63).

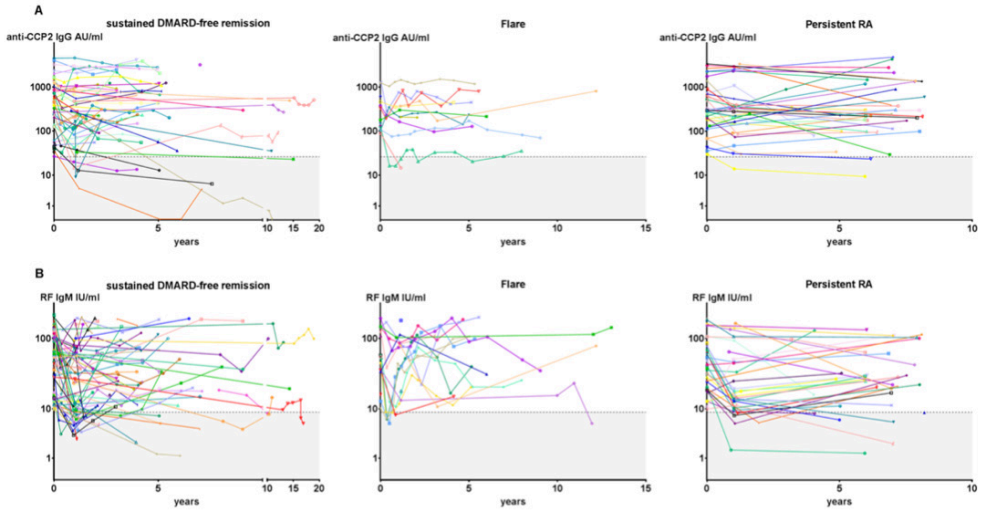

Figure 9.3 Anti-CCP2 IgG levels in ACPA-positive RA patients (A) and RF IgM levels in RF-positive patients (B) during follow-up, stratified for clinical outcome

Dotted lines indicate the cut-off values (25 AU/mL for anti-CCP2 IgG and 8 IU/mL for RF IgM). Values below the detection limit were imputed with the value of 0. Number of ACPA and RF-positive patients in each group: DMARD-free sustained remission: ACPA+ n=54, RF+ n=86, flare: ACPA+ n=15, RF+ n=21, persistent RA: ACPA+ n=35, RF+ n=41. ACPA, anti-citrullinated protein antibodies; anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

Patients with seroreversion had lower median anti-CCP2 IgG levels at disease presentation than patients without seroreversion (42 and 420 AU/mL, respectively, p<0.001). Ever use of biological DMARDs was comparable in patients with and without seroreversion (33% and 32%, respectively, p=1.00). To further analyse the effect of differences in medication use between patients, analyses were stratified for medication ever used during follow-up. This revealed similar results as in the whole group of patients (Supplementary table 9.1).

Similar results were observed for RF IgM (Figures 9.3B and 9.4B). RF-positive patients who achieved sustained DMARD-free remission had seroreversion in 20%, whereas this occurred in 6% and 15% of patients with a late flare and with persistent disease, respectively (p=0.44, Figure 9.4B). RF IgM levels were lower in patients with seroreversion than in patients who remained positive for RF IgM (19 and 53 IU/mL, respectively, p=0.003). Thus, ACPA or RF seropositive RA patients who achieved sustained DMARD-free remission did not become more frequently seronegative than patients who did not achieve remission.

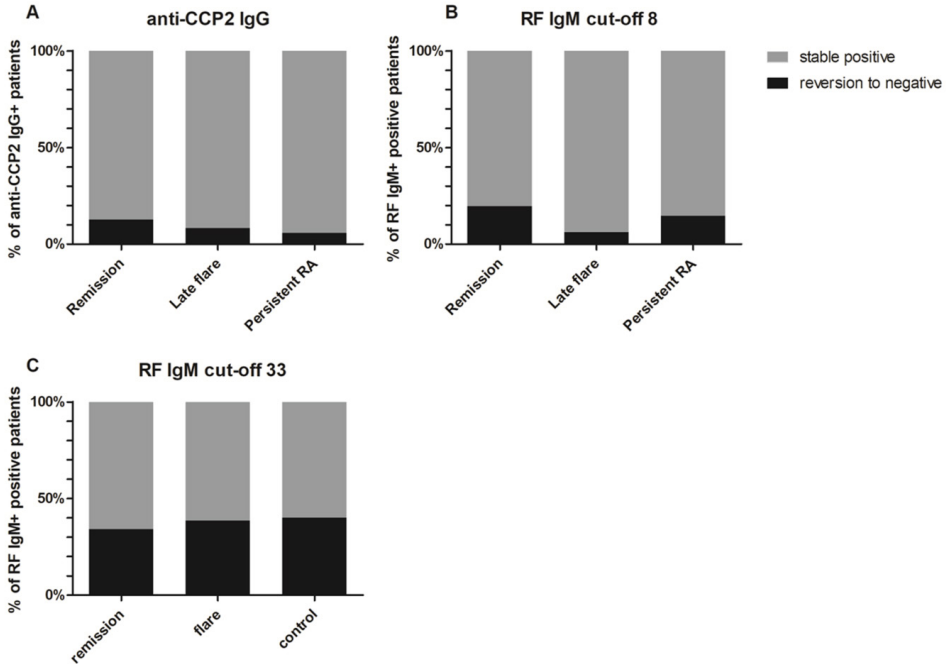

Figure 9.4 Reversion to anti-CCP2 IgG (A) and RF IgM (B, C) seronegativity in autoantibody-positive RA patients who achieved sustained DMARD-free remission, who had a late flare and in patients with persistent RA

Analyses were performed in patients positive for anti-CCP2 IgG (A) or positive for RF IgM (B, C). For the analyses presented in (A) and (B) the cut-offs used were determined by the manufacturer and are similar to those used in clinical practice. For RF, also a cut-off of 33 was used to define positivity. This cut-off resulted in a specificity of 98% relative to patients with other early arthritides and was then comparable to the ACPA test (C). Seroreversion was defined as shifting from seropositive at baseline to seronegative in the last available serum sample; for patients who achieved sustained DMARD-free remission the last sample was measured at the time of remission. Number of patients in each group: sustained DMARD-free remission: ACPA+ n=47, RF+ cut-off 8 n=71, RF+ cut-off 33 n=44, late flare: ACPA+ n=12, RF+ cut-off 8 n=16, RF+ cut-off 33 n=13, persistent RA: ACPA+ n=35, RF+ cut-off 8 n=41, RF+ cut-off 33 n=25. ACPA, anti-citrullinated protein antibodies; anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

Changes in RF IgM levels were larger in patients with sustained DMARD-free remission than in patients with persistent RA

Next, it was evaluated whether changes in autoantibody levels during the total follow-up period differed between patients. The change in anti-CCP2 IgG level per year was not associated with achievement of sustained DMARD-free remission (p=0.66). For RF IgM-positive patients, the change in RF level was associated with achievement of sustained DMARD-free remission; for every 10-unit decrease in RF IgM level per year the rate of sustained DMARD-free remission increased by

16% (p<0.001). Thus, seropositive RA patients who achieved sustained DMARD-free remission did not have disappearance of autoantibodies; however, there was a significant decrease of RF IgM levels in patients who achieved sustained DMARD-free remission compared with patients who did not.

Anti-CCP2 IgM seroreversion was not associated with sustained DMARD-free remission

Finally, the proportion of patients seroreverting from anti-CCP2 IgM positive to negative was studied as we hypothesised that if the ACPA immune response had changed in patients who achieved remission, this could be reflected by a decreased presence of anti-CCP2 IgM, since IgM is an indication of an ongoing immune response. Of anti-CCP2 IgG-positive patients, 25-29% were also positive for anti-CCP2 IgM at disease presentation within the different groups. During follow-up, 31% (4/13) of the anti-CCP2 IgG and IgM-positive patients who achieved DMARD-free remission seroreverted from positive to negative anti-CCP2 IgM (Figure 9.5). For patients with a late flare and patients with persistent RA, this occurred in 100% (3/3) and 60% (6/10), respectively. Thus, patients who achieved sustained DMARD-free remission and who were seropositive for anti-CCP2 IgM at disease presentation did not serorevert more frequently than patients who did not achieve remission.

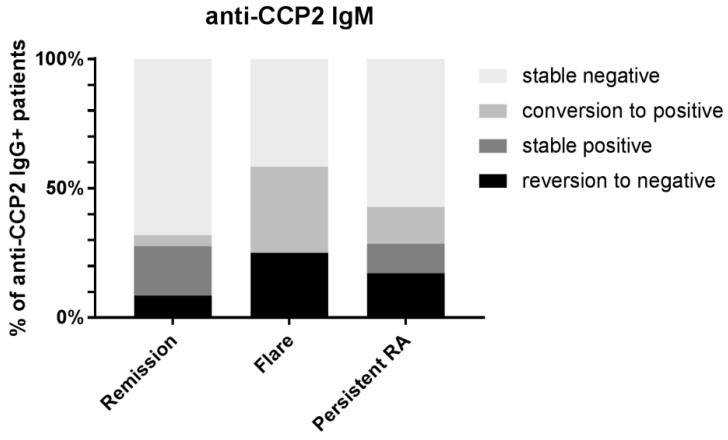

Figure 9.5 Change in anti-CCP2 IgM status during follow-up in patients who achieved DMARD-free sustained remission, who had a flare and in patients with persistent RA

Data are shown for the subgroup of anti-CCP2 IgG+ patients at baseline. Number of patients in each group: DMARD-free sustained remission: n=47, flare: n=12, persistent RA: n=35. Anti-CCP2, anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis.

Sensitivity analyses

To investigate whether our results were driven by patients with autoantibody levels fluctuating around the cut-off, analyses were performed in patients with baseline autoantibody levels above the median which showed that none of the anti-CCP2 IgG-positive patients had seroreverted. For RF IgM, 8% of patients who achieved sustained DMARD-free remission, none of the patients with a late flare and 10% of patients with persistent disease had seroreverted.

To study the hypothesis that early treatment might be associated with higher rates of seroreversion, analyses were repeated in patients with short symptom duration before treatment start (<12 weeks) and with long symptom duration (≥12 weeks). Of anti-CCP2 IgG-positive patients who achieved remission, 10% of the patients with a short symptom duration had reverted to seronegativity; this was 15% of patients with a long symptom duration (p=1.00). For RF IgM, these percentages were 25% and 18%, respectively (p=0.54). Thus, seroreversion rates were not higher in patients with earlier treatment initiation.

Next, it was assessed whether single autoantibody positivity was associated with higher seroreversion rates. Of ACPA-positive patients, 7% of ACPA+ RF+ and 23% of ACPA+ RF- patients had reverted from anti-CCP2 IgG positivity to negativity (p=0.12). Of RF-positive patients, 6% of ACPA+ RF+ and 34% of ACPA- RF+ patients had reverted to RF IgM negativity (p<0.001).

In addition, analyses were stratified for different autoantibody combinations, since patients with sustained DMARD-free remission were less frequently positive for both ACPA and RF than the other studied groups. Similar to our main analysis, seroreversion rates for ACPA and RF were not significantly different between patients with and without sustained remission (Supplementary table 9.2). Thus, single RF-positive patients seroreverted more often (when cut-off of 8 was used) than ACPA+ RF+ patients, but the frequency of seroreversion was not higher in the group that achieved sustained remission group compared with the group that did not.

Analyses were also repeated in patients who had a follow-up duration of ≥4.2 years after achievement of sustained DMARD-free remission (i.e., within 50% of patients with the longest follow-up after DMARD cessation). This analysis was performed to verify if patients with shorter follow-up after DMARD cessation influenced the results, as these patients could be at risk of developing a late flare. Of anti-

CCP2 IgG-positive RA patients who achieved sustained DMARD-free remission, 6% had reverted to anti-CCP2 negativity around the time of remission, which was not different from patients with a late flare and with persistent disease, who had seroreversion in 8% and 6%, respectively (p=1.00). Of RF IgM-positive RA patients who achieved sustained DMARD-free remission, 16% had reverted to RF IgM negativity. Of patients with a late flare and with persistent disease this occurred in 6% and 15%, respectively (p=0.78).

Finally, seroreversion for RF was also studied when the cut-off for positivity was set at 33. Also then no differences in seroreversion rates were observed between the three different groups (p=0.86, Figure 9.4C). When the different autoantibody combinations were studied with this cut-off, 6% of ACPA+ RF+ and 16% of ACPA+ RF- patients had reverted to anti-CCP2 negativity (p=0.27). Of RF-positive patients, 37% of ACPA+ RF+ and 35% of ACPA- RF+ patients had reverted to RF IgM negativity (p=1.00). Thus, also when a different cut-off for RF positivity was used, patients who achieved sustained DMARD-free remission did not serorevert more often than patients with a late flare or with persistent disease.

Discussion

Currently, a sustained DMARD-free status is the best possible clinical outcome as, per definition, clinically apparent synovitis is persistently absent and patients in this status also have resolution of symptoms and normalised functional status.[1] This outcome is achievable in autoantibody-positive RA, although with a lower frequency than in autoantibody-negative RA. The biological nature underlying this type of persistent remission is unknown. It was recently suggested that it is characterised by disappearance of autoantibodies.[14] The present large observational study with a unique, long follow-up period and with samples measured at the time of remission, explored this hypothesis. No association between remission and reversion to autoantibody negativity was demonstrated. Hence, although it has been suggested that immunological remission is characterised by disappearance of autoantibodies,[14] we studied patients in the deepest form of clinical remission and observed no increased frequency of reversion to seronegativity in this group.

To our knowledge, this is the first study in which seroreversion rates in patients with long-standing DMARD-free status were investigated. Importantly, for both ACPA and RF seroreversion was infrequent and not related to clinical outcome. In

previous studies in patients with established RA who were treated with DMARDs and thus had persistent disease, similar seroreversion rates were observed.[18,25-27] Thus, seroreversion rates observed here are in line with previous findings in RA and are not increased in patients with sustained DMARD-free status.

When analyses were repeated in patients with high autoantibody levels (above the median) at baseline, seroreversion for anti-CCP2 IgG was not observed anymore, and seroreversion for RF IgM was less frequent than in the whole group of autoantibody-positive patients. This suggests that the observed seroreversion rates were mainly the result of patients who fluctuated around the cut-off level, and therefore that true seroreversion of ACPA and RF is only sporadically observed.

Although no association between remission and seroreversion was observed, patients who achieved a DMARD-free status had a larger decrease in RF levels during follow-up than patients who did not achieve this outcome. The slopes of the levels along follow-up are relevant to appreciate the immunological evolution, which was different for RF and ACPA. Several studies have shown that improvement in disease activity is accompanied by decrease in RF levels,[27-31] although some other studies did not observe this.[18,19,32] A unique feature of this study is that a prolonged period of absence of clinical synovitis is observed, which is a much more stringent outcome than improvement in disease activity scores. Previous studies showed no relation between ACPA levels and disease activity and our data also demonstrated no relation with sustained DMARD-free status.[27-31]

Previously, it was shown that anti-CCP2 IgM remains present in RA patients during treatment in a persistent disease phase, suggesting that the anti-CCP immune response is continuously reactivated.[33] Interestingly, we have shown here that anti-CCP2 IgM also remained persistently present in patients who achieved sustained DMARD-free remission. This suggests that even in patients who are clinically cured, the anti-CCP response is persistently activated. ACPA characteristics other than level and IgM and IgG isotypes were not investigated. However, it is known that ACPA-level is highly associated with ACPA fine specificity and the number of ACPA isotypes.[34] Based on this, it can be presumed that these characteristics were also not different between patients who did or did not achieve a sustained DMARD-free status. Nonetheless, we do not rule out that these, or other characteristics of the autoantibody response in RA correlate with the induction of sustained DMARD-free remission. Altogether, disappearance of clinical disease is not accompanied by changes in the humoral ACPA response in serum. Thus,

whereas for RA development it is not yet elucidated whether ACPA play a role in the pathophysiology or act as bystander, the present data suggest that the ACPA response does not explain the maintained resolution of clinical disease. Whether other immunological markers, for instance, changes in characteristics of autoantibody expressing B cells, associate with this phenotypic outcome, and therefore would be a better definition of immunological remission, is subject of further research.

A strength of this study is that patients had a long follow-up period also after DMARD cessation (median 4.2 years after achievement of sustained DMARD-free remission, thus median 5.2 years after DMARD stop). This follow-up time supports the validity of the outcome, as patients with an early flare after DMARD stop were never considered to be in remission, and also patients with a late flare were identified and excluded from the group of patients who achieved sustained DMARD-free remission. Late flares generally occurred 2.2 years after DMARD cessation and the large majority of patients were followed for a longer period of time after DMARD stop. A subanalysis in the patients who were followed for >4 years after achieving remission showed similar results, showing the robustness of the data in this respect. Of course, we do not know if the autoantibody-positive patients will get a flare of disease after an even longer follow-up period; this is subject of future studies. Some of the patients have been discharged from the outpatient clinic because of prolonged absence of synovitis and symptoms. Importantly, it is plausible that if symptoms will recur patients will return to our clinic since the Leiden University Medical Center is the only referral centre in a healthcare region of ~400,000 inhabitants and has very easy access services, allowing that patients with symptoms suspicious of RA are seen within 1 week.[35]

Treatment was not protocolised and the applied treatments changed over time. This resulted in heterogeneity of treatments received by patients. Nonetheless, when stratifying patients in groups according to medications ever used during follow-up, results remained similar.

Another limitation might be that not of all patients with sustained DMARD-free remission serum samples were available after medication was stopped. However, when analyses were repeated in this subgroup of patients, similar results were obtained (data not shown).

Previously, it was suggested that remission can be defined according to different

conditions and presence of immunological remission, defined as the disappearance of autoantibodies, was suggested to be the deepest form of remission. In this long-term study, we were able to analyse a large number of ACPA-positive patients who achieved sustained DMARD-free remission. In this unique dataset, we observed that disappearance of autoantibodies rarely occurred, and that patients who achieved the best possible outcome of RA did not become more often seronegative than patients with persistent disease. Therefore, in our view, this definition of immunological remission should not be a long-term treatment target.

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Supplementary material

Supplementary table 9.1 Comparison of seroreversion rates between patients with sustained DMARD-free remission and with persistent RA, stratified for medication use during follow-up

		DMARD-free sustained remission	Flare after DMARD-free remission	Persistent RA	p-value (Fisher exact)
Methotrexate+ ACPA+ n=90	anti-CCP2 IgG seroreversion, n(%)	6 (14)	1 (8)	2 (6)	0.64
Methotrexate+ RF+ n=116	RF IgM seroreversion, n(%)	10 (17)	1 (6)	6 (15)	0.71
Sulfasalazine+ ACPA+ n=41	anti-CCP2 IgG seroreversion, n(%)	3 (19)	0 (0)	1 (5)	0.48
Sulfasalazine+ RF+ n=48	RF IgM seroreversion, n(%)	3 (16)	0 (0)	4 (16)	1.00
Hydroxychloroquine+ ACPA+ n=43	anti-CCP2 IgG seroreversion, n(%)	2 (18)	0 (0)	1 (4)	0.31
Hydroxychloroquine+ RF+ n=58	RF IgM seroreversion, n(%)	6 (26)	0 (0)	1 (3)	0.05
TNF-inhibitor + ACPA+ n=23	anti-CCP2 IgG seroreversion, n(%)	2 (25)	1 (100)	0 (0)	0.02
TNF-inhibitor + RF+ n=26	RF IgM seroreversion, n(%)	0 (0)	0 (0)	2 (13)	0.63

Patients were stratified for medication use during follow-up. The duration of treatment and combination therapy is not indicated here. Numbers indicate the number of patients with CCP2 IgG or RF IgM seroreversion within patients who where ACPA-positive or RF-positive at disease presentation, and who used the indicated medication during follow-up. ACPA, anti-citrullinated protein antibodies; anti-CCP anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumor necrosis factor.

Supplementary table 9.2 Comparison of seroreversion rates between patients with sustained DMARD-free remission and with persistent RA, stratified for baseline autoantibody status

		Sustained DMARD-free remission	No sustained DMARD-free remission	p-value (Fisher exact)
A	**ACPA+RF+**			
	anti-CCP2 IgG reversion to negative, n(%)	4 (11)	2 (5)	0.41
	anti-CCP2 stable positive, n(%)	34 (89)	41 (95)	
	RF IgM reversion to negative, n(%)	4 (11)	1 (2)	0.18
	RF IgM stable positive, n(%)	34 (89)	42 (98)	
	ACPA+RF-			
	anti-CCP2 IgG reversion to negative, n(%)	2 (22)	1 (25)	1.00
	anti-CCP2 stable positive, n(%)	7 (78)	3 (75)	
	ACPA-RF+			
	RF IgM reversion to negative, n(%)	10 (30)	6 (43)	0.51
	RF IgM stable positive, n(%)	23 (70)	8 (57)	
B	**ACPA+RF+**			
	anti-CCP2 IgG reversion to negative, n(%)	2 (7)	2 (6)	1.00
	anti-CCP2 stable positive, n(%)	28 (93)	30 (94)	
	RF IgM reversion to negative, n(%)	11 (37)	12 (37.5)	1.00
	RF IgM stable positive, n(%)	19 (63)	20 (62.5)	
	ACPA+RF-			
	anti-CCP2 IgG reversion to negative, n(%)	4 (24)	1 (7)	0.34
	anti-CCP2 stable positive, n(%)	13 (76)	14 (93)	
	ACPA-RF+			
	RF IgM reversion to negative, n(%)	4 (29)	3 (50)	0.61
	RF IgM stable positive, n(%)	10 (71)	3 (50)	

Patients were stratified for autoantibody status at baseline. In part A the cut-off for RF positivity was 8 IU/ml, in part B this was 33 IU/ml to arrive at a specificity of 98%, comparable to the ACPA test. Numbers indicate the number of patients with CCP2 IgG or RF IgM seroreversion within patients who where ACPA-positive or RF-positive at disease presentation. ACPA, anti-citrullinated protein antibodies; anti-CCP anti-cyclic citrullinated peptide 2; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; RF, rheumatoid factor.

10

Subdividing ACPA-negative RA: patients with high likelihood of achieving sustained DMARD-free remission are characterized by a combination of serological markers at disease presentation

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Abstract

Background

Disease-modifying antirheumatic drug (DMARD)-free remission, the sustained absence of synovitis after DMARD cessation, is increasingly achievable, especially in autoantibody-negative rheumatoid arthritis (RA). However, underlying mechanisms are unknown and patient subgroups that achieve this outcome are insufficiently characterized. We evaluated whether serological biomarkers at disease onset, as measured within the multibiomarker disease activity (MBDA) score, are differently expressed in RA patients who achieve sustained DMARD-free remission.

Methods

Two hundred ninety-nine RA patients were evaluated for achievement of sustained DMARD-free remission during a median follow-up of 4.3 years. Twelve biomarkers, as included in the MBDA score, were determined from the serum obtained at disease onset. Patients were categorized as having a low (<30), moderate (30-44) or high (>44) score. Analyses were stratified for anti-citrullinated protein antibodies (ACPA) based under the assumption that ACPA-positive and ACPA-negative RA are different disease entities.

Results

Twenty percent achieved sustained DMARD-free remission. Overall, high MBDA scores were associated with achieving DMARD-free remission (high vs. low HR 3.8, 95% CI 1.2-12.2). Among ACPA-negative RA patients, moderate or high scores associated strongly with DMARD-free remission (moderate vs. low HR 9.4, 95% CI 1.2-72.9; high vs. low HR 9.7, 95% CI 1.3-71.1). This association was independent of age and other clinical factors (high vs. low HR 8.2, 95% CI 1.1-61.8). For ACPA-negative RA patients, the biomarkers C-reactive protein, serum amyloid A and matrix metalloproteinase-3 were individually associated with sustained DMARD-free remission. Among ACPA-positive RA patients, scores were not associated with DMARD-free remission.

Conclusions

ACPA-negative RA patients who achieved sustained DMARD-free remission after treatment withdrawal were characterized by moderate to high MBDA scores at diagnosis. This is the first evidence that ACPA-negative RA can be subdivided in clinically relevant subsets at disease onset using a protein profile.

Background

Rheumatoid arthritis (RA) is a syndrome which presumably consists of several disease entities. Most data have focused on differences in RA characterized by the presence and absence of autoantibodies, in particular anti-citrullinated protein antibodies (ACPA). ACPA-positive patients have in general a more persistent and destructive disease course than ACPA-negative patients. The generation of different disease subsets in seronegative patients that have a clinical diagnosis of RA and fulfil respective classification criteria is unsuccessful thus far.[1] Therefore, we investigated if we could identify patients in the ACPA-negative subgroup that have the best clinical outcome, which currently is the achievement of sustained disease-modifying antirheumatic drug (DMARD)-free remission.

The biological mechanisms underlying the achievement of sustained DMARD-free remission are unknown. Additionally, it is undefined whether this outcome is potentially achievable by all RA patients or whether the ability to permanently stop DMARDs is restricted to a set of RA patients with certain biological characteristics. Several studies have shown that a shorter symptom duration, which is a disease phase characteristic rather than a 'patient characteristic', is associated with a greater probability of achieving sustained DMARD-free remission.[2-6] The second important factor is the absence of ACPA.[1,2,6] This suggests that patients who can achieve remission are inherently different. However, the absence of autoantibodies only explains part of the variability in outcome, since a proportion of ACPA-positive patients can achieve sustained DMARD-free remission and the majority of ACPA-negative patients do not achieve it.[7] We assumed that patients who are able to achieve sustained DMARD-free remission are intrinsically different from patients who are unable to do so. If this hypothesis is true, these patients might be identifiable by biomarkers present at disease presentation. With respect to systemically measurable markers, C-reactive protein (CRP) has been studied and decreased levels were associated with sustained DMARD-free remission in one study,[2] while in another study no association was observed.[8] Other inflammatory proteins have not been studied in relation to sustained DMARD-free remission.

Several serological biomarkers are combined in the multi-biomarker disease activity (MBDA) score, which is developed to measure RA disease activity.[9,10] The level of the 12 biomarkers which are combined in the MBDA score might indicate relevant pathways involved in RA disease activity, and the combination of markers may provide more information than markers such as the erythrocyte sedimentation

rate (ESR) or CRP alone. Several studies have shown that higher MBDA scores measured during the disease course are predictive of radiographic progression in the next years,[11-13] although there are also studies showing no association.[14-16] It is unexplored if the serological biomarkers included in the score are associated with an opposite, favourable outcome, i.e. achieving sustained DMARD-free remission.

Our ultimate aim was to identify subgroups of RA patients that are identifiable at disease presentation, for which sustained DMARD-free remission is an achievable outcome. We hypothesized that individual serological markers or a combination of these is helpful to characterize these subgroups. Therefore, we investigated the association between the MBDA score and its component serological markers at first presentation with RA and the achievement of sustained DMARD-free remission. We observed that the subgroup of ACPA-negative RA patients with a high chance of achieving sustained DMARD-free remission can already be identified at the time of diagnosis by the presence of a combination of proteins.

Methods

Patients

The Leiden Early Arthritis Clinic cohort is an inception cohort that enrolls patients with clinically confirmed arthritis of recent onset and symptom duration <2 years. At baseline, questionnaires were administered, joint counts and blood samples were collected and patients were evaluated annually thereafter.[1] Baseline serum samples were tested for CRP level, ESR, IgG ACPA (EliA CCP (anti-CCP2), Phadia, Nieuwegein, the Netherlands) and IgM rheumatoid factor (RF; in-house ELISA, as described previously).[17] Patients did not use DMARDs or glucocorticoids before inclusion.

For this study, RA patients included between 2010 and 2015 were evaluated, since this is the most recent inclusion period and since we have shown that sustained DMARD-free remission is increasingly achievable with current treatment strategies.[8] RA was stringently defined by a clinical diagnosis of RA by an experienced rheumatologist. Besides a clinical diagnosis, patients needed to fulfil the 1987 or 2010 classification criteria during the first year.[18,19] Both classification criteria were considered since ACPA-negative patients can be misclassified by the 2010 criteria because they need >10 involved joints to achieve 6 points. Thus, all included RA patients had a clinical diagnosis of RA and in addition fulfilled RA

classification criteria. Patients diagnosed with conditions other than RA during the follow-up were not included in this study. In the period mentioned, 321 patients were eligible. Thirteen patients were excluded because they did not use DMARDs during the follow-up and 9 because measurement of an MBDA biomarker had failed. Thus, in total, 299 patients were studied.

The initial treatment of RA consisted of methotrexate, which could be combined with low-dose prednisone bridging therapy at DMARD start. Typically, when the first treatment failed, another conventional DMARD was initiated or added. A biological DMARD was allowed in patients that failed on ≥2 conventional DMARDs. During the full observation period, 91% of patients ever used methotrexate, 85% ever used other conventional DMARDs (systemic glucocorticoids, sulfasalazine, hydroxychloroquine, leflunomide or azathioprine) and 20% ever used biologicals. ACPA-positive patients more frequently used biologicals; further details are shown in Supplementary table 10.1. According to local and international guidelines, treatment was DAS44 guided with DMARD tapering in case of a DAS <2.4 and intensifying in case of a DAS ≥2.4.[20] Subsequent to DMARD tapering, DMARDs were stopped in case the DAS44 remained <2.4 and synovitis was absent at clinical joint examination. Thereafter, patients were followed on the recurrence of synovitis or persistence of DMARD-free remission. The study was approved by the local medical ethics committee, and all patients signed informed consent.

Sustained DMARD-free remission

Medical files were reviewed for all patients until April 2017 to identify the occurrence of sustained DMARD-free remission, which was defined as the absence of synovitis (by physical examination) that sustained after discontinuation of all DMARD therapy (including biologics and systemic and intra-articular corticosteroids) for the entire follow-up period and must have extended to at least 1 year after DMARD withdrawal. The date of sustained DMARD-free remission was defined as the date 1 year after DMARDs were stopped. Patients who did not achieve remission were censored at the date when the medical file was explored or when they were lost to follow-up. One patient achieved sustained DMARD-free remission but relapsed during follow-up and was considered as not in remission.

The MBDA score

Serum samples were collected at disease presentation, before any DMARD treatment (including glucocorticoids) was started, and stored at -80 °C. Crescendo Bioscience (South San Francisco, CA, USA) measured concentrations of 12 biomarkers using

three separate multiplex, sandwich immunoassays: CRP, IL-6 (interleukin-6), SAA (serum amyloid A), TNFR1 (tumor necrosis factor receptor superfamily member 1A), EGF (epidermal growth factor), VEGF-A (vascular endothelial growth factor-A), VCAM-1 (vascular cell adhesion molecule-1), MMP-1 (matrix metalloproteinase-1), MMP-3 (matrix metalloproteinase-3), YKL-40 (human cartilage glycoprotein-39), resistin and leptin. Measurements were performed blinded to clinical data and outcome. The biomarkers were studied individually and in combination by using a previously specified algorithm to calculate the MBDA score, ranging on a scale from 1 to 100.[9,10,21] This MBDA algorithm was developed to measure disease activity with DAS28-CRP as reference. For analyses, patients were categorized according to previously established thresholds in categories of low (<30), moderate (30-44) and high (>44) MBDA score.[10] Although we used the MBDA score for a purpose different than measuring disease activity, we used the same cut-off points for categorization.

Statistical analyses

Kaplan-Meier analysis was used to estimate rates of achieving sustained DMARD-free remission with MBDA category and the 12 individual biomarkers as grouping factors. For the latter analyses, patients were categorized into tertiles based on the biomarker levels to create three groups of equal size. Univariable Cox proportional hazards regression analyses were used to assess the association between baseline characteristics and the achievement of sustained DMARD-free remission. Baseline variables with a p-value <0.10 were included in a multivariable analysis to assess the independent relation between the serological markers and the achievement of sustained DMARD-free remission. Because achieving sustained DMARD-free remission is mostly confined to ACPA-negative RA and since we aimed to search for subgroups within ACPA-negative and ACPA-positive RA, analyses were stratified for the presence of ACPA. SPSS version 23.0 (IBM) was used. P-values <0.05 were considered significant.

Results

Patient characteristics

Baseline characteristics of the 299 RA patients are presented in Table 10.1. The median symptom duration at first presentation was 15 weeks (interquartile range (IQR) 8-32) and, similar to other early arthritis cohorts, 53% of patients were ACPA-positive.[22,23]

Table 10.1 Baseline characteristics of all RA patients and of subgroups of ACPA-positive and ACPA-negative patients

	all RA patients (n=299)	ACPA-positive RA patients (n=158)	ACPA-negative RA patients (n=141)
Age in years, mean (SD)	57 (14)	54 (14)	60 (14)
Female, n (%)	198 (66)	105 (66)	93 (66)
Symptom duration in weeks, median (IQR)	15 (8-32)	18 (9-38)	12 (5-26)
(Sub)acute symptom onset, n(%)	95 (34)	39 (27)	56 (43)
66-SJC, median (IQR)	6 (3-11)	5 (2-8)	8 (3-12)
68-TJC, median (IQR)	9 (4-15)	7 (4-13)	10 (4-18)
RF positivity, n (%)	183 (61)	134 (85)	49 (35)
ESR (mm/h), median (IQR)	28 (14-41)	28 (14-41)	28 (11-41)
CRP (µg/ml), median (IQR)	10 (3-23)	8 (3-18)	12 (3-30)
PTGA (0-100), median (IQR)	70 (50-80)	70 (45-80)	70 (60-80)
DAS44, median (IQR)	2.9 (2.4-3.4)	2.8 (2.3-3.3)	3.0 (2.5-3.7)
MBDA category			
low (<30), n (%)	43 (14)	26 (16)	17 (12)
moderate (30-44), n (%)	64 (21)	35 (22)	29 (21)
high (>44), n (%)	192 (64)	97 (61)	95 (67)

Some data were missing as follows: symptom duration n=4, (sub)acute symptom onset n=23, 66-SJC n=19, 68-TJC n=17, ESR n=3, PTGA n=50 , DAS44 n=20 and CRP n=1. ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; IQR, interquartile range; MBDA, multibiomarker disease activity; PTGA, patient global assessment; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; (sub)acute symptom onset, prompt onset of symptoms (<1 week); SJC, 66-swollen joint count; symptom duration, time between symptom onset and inclusion in cohort; TJC, 68-tender joint count.

Development of sustained DMARD-free remission and distribution of MBDA scores

The median follow-up duration was 4.3 years (IQR 4.0-4.7). Sustained DMARD-free remission was achieved in 20% (59/299) of RA patients after a median follow-up of 2.9 years (IQR 2.2-4.0). Sustained DMARD-free remission was achieved by 7% (11/158) of ACPA-positive patients and 34% (48/141) of ACPA-negative patients.

A combination of serological markers as reflected by MBDA scores associated with sustained DMARD-free remission within ACPA-negative RA

First, the association between the achievement of sustained DMARD-free remission during follow-up and the MBDA score at disease onset was evaluated in all RA patients (Figure 10.1A). With patients with low MBDA scores as reference, patients with moderate MBDA scores had an increased probability on the development of sustained DMARD-free remission (HR 3.42, 95% CI 0.97-12.02). A similar increased probability was observed for patients with high MBDA scores (HR 3.79, 95% CI 1.18-12.22). Next, patients were stratified for the presence of ACPA (Figure 10.1B, C). For

ACPA-positive RA patients, the baseline MBDA category was not associated with achieving sustained DMARD-free remission (moderate vs. low HR 0.75, 95% CI 0.10-8.19; high vs. low HR 0.89, 95% CI 0.19-4.31). By contrast, among ACPA-negative RA patients, moderate or high MBDA scores were strongly associated with achieving sustained DMARD-free remission (moderate vs. low HR 9.40, 95% CI 1.21-72.85; high vs. low HR 9.73 95% CI 1.33-71.10). Sustained DMARD-free remission was almost absent in the ACPA-negative group with low MBDA scores (only one patient in this group achieved remission after 6 years follow-up), whereas sustained DMARD-free remission was achieved by 38% of the ACPA-negative patients with moderate or high MBDA scores. The HR for achieving remission was 9.65 (95% CI 1.33-70.04) when ACPA-negative RA patients with either moderate or high MBDA scores were compared with patients with low MBDA scores. Thus, only for ACPA-negative RA patients, a combination of serological markers at diagnosis, reflected by the MBDA score, was associated with achievement of sustained DMARD-free remission.

A combination of serological markers associated with sustained DMARD-free remission, independent of clinical factors

Next, we investigated whether the association between baseline MBDA score and sustained DMARD-free remission within ACPA-negative patients was independent of clinical characteristics. Of the clinical baseline characteristics, age at disease onset, the 66-swollen joint count and the presence of RF associated with sustained DMARD-free remission, with a p-value <0.10 in ACPA-negative RA; these characteristics were included in a multivariable analysis (Table 10.2). In this analysis, the MBDA category was associated with sustained DMARD-free remission, independent of these three factors, with moderate vs. low HR 6.96 (95% CI 0.88-55.31) and high vs. low HR 8.19 (95% CI 1.09-61.78) (Table 10.2).

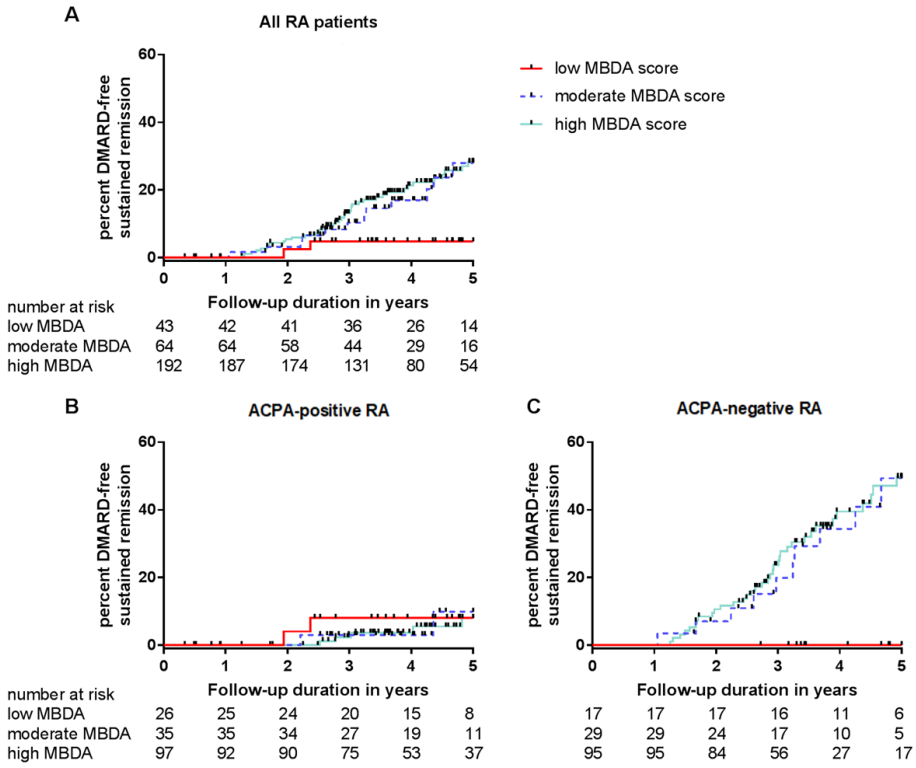

Figure 10.1 Kaplan-Meier plot showing achievement of sustained DMARD-free remission by category of MBDA score for all RA patients (A), ACPA-positive RA patients (B) and ACPA-negative RA patients (C)

Vertical lines indicate that a patient is censored. The numbers below the figures denote the number of patients at risk in each group. Visual representation of the data was restricted to 5 years follow-up since thereafter the number of patients was small. ACPA, anti-citrullinated protein antibodies; DMARD, disease-modifying antirheumatic drug; MBDA, multi-biomarker disease activity; RA, rheumatoid arthritis.

Among ACPA-negative RA patients, higher CRP, SAA and MMP-3 levels associated with achieving sustained DMARD-free remission

Next, it was studied whether the observed association for ACPA-negative RA patients was driven by a subset of markers of the MBDA score. Therefore, the association between the level of the 12 individual biomarkers included in the MBDA score and the achievement of sustained DMARD-free remission was determined for ACPA-negative RA patients (Supplementary figure 10.1). Of the individual biomarkers, higher CRP, SAA and MMP-3 levels at disease onset were associated with achieving sustained DMARD-free remission during the follow-up. Patients with CRP levels

7-39 µg/mL (second tertile) had a significantly increased probability on achieving sustained DMARD-free remission compared with patients with CRP levels <7 µg/mL (lowest tertile) (HR 3.43, 95% CI 1.62-7.27), and for patients with CRP levels ≥39 µg/mL (highest tertile), a similar trend was observed (HR 2.12, 95% CI 0.96-4.70). In addition, patients with MMP-3 levels ≥60 ng/mL (highest tertile) had a significantly increased probability on the development of sustained DMARD-free remission compared with patients with MMP-3 levels ≤28 ng/mL (lowest tertile) (HR 2.18, 95% CI 1.06-4.48). SAA levels were also associated with achieving DMARD-free sustained remission. Patients with SAA levels ≥26 µg/mL (highest tertile) or 3-26 µg/mL (second tertile) had a significantly increased probability on the development of sustained DMARD-free remission compared with patients with SAA levels ≤3 µg/mL (lowest tertile) (HR 2.87, 95% CI 1.28-6.43 and HR 3.03, 95% CI 1.39-6.63, respectively). The other biomarkers were not individually associated with the achievement of sustained DMARD-free remission.

Table 10.2 Association between the MBDA score and achieving sustained DMARD-free remission over time within ACPA-negative RA patients

	ACPA-negative RA patients					
	Univariable analyses		Multivariable analysis Without MBDA		Multivariable analysis Including MBDA	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
MBDA score						
low	reference				reference	
moderate	9.40 (1.21-72.85)	0.032			6.96 (0.88-55.31)	0.067
high	9.73 (1.33-71.10)	0.025			8.19 (1.09-61.78)	0.041
Age at disease onset, per year	1.04 (1.02-1.07)	0.001	1.03 (1.01-1.06)	0.006	1.03 (1.00-1.06)	0.036
Female	1.43 (0.80-2.55)	0.23				
Symptom duration >12 weeks	0.96 (0.54-1.70)	0.89				
(Sub)acute symptom onset	0.93 (0.51-1.69)	0.81				
66-SJC, per joint	1.04 (0.99-1.08)	0.099	1.03 (0.99-1.08)	0.15	1.02 (0.98-1.06)	0.40
68-TJC, per joint	0.99 (0.96-1.02)	0.56				
ESR, per mm/h	1.01 (1.00-1.02)	0.17				
CRP, per mg/L	1.00 (1.00-1.01)	0.36				
RF positivity	0.57 (0.29-1.09)	0.088	0.84 (0.42-1.66)	0.61	0.78 (0.39-1.58)	0.50

Of the 141 ACPA-negative RA patients, 48 achieved sustained DMARD-free remission. Baseline variables with a p-value <0.10 in univariable analyses were included in a multivariable analysis to assess the independent relation between baseline variables and sustained DMARD-free remission. ACPA, anti-citrullinated protein antibodies; CI, confidence interval; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HR, hazard ratio; MBDA, multi-biomarker disease activity; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, 66-swollen joint count; (sub)acute symptom onset, prompt onset of symptoms (<1 week); symptom duration, time between symptom onset and inclusion in cohort; TJC, 68-tender joint count.

Clinical characteristics at disease presentation of ACPA-negative RA patients with an elevated MBDA score

Finally, as ACPA-negative RA patients with a high probability on achieving sustained DMARD-free remission were identifiable by a protein profile that reflected high disease activity at the time of diagnosis, we evaluated whether these patients also had differences in clinical characteristics compared with those presenting with low MBDA scores. ACPA-negative patients with a high MBDA score more often had a subacute onset of symptoms (vs. gradual or intermittent onset) (Table 10.3). ACPA-negative patients with a moderate or high MBDA score were approximately 10 years older and had higher acute phase reactants at the first presentation, compared with ACPA-negative patients with a low MBDA score (Table 10.3). These associations with clinical characteristics at diagnosis suggest that subgroups of ACPA-negative RA, differentiated based on serological biomarkers, not only have differences in long-term outcome but also differ already at disease presentation.

Table 10.3 Baseline characteristics of ACPA-negative RA patients per MBDA category

	MBDA category			p-value
	<30 (n=17)	30-44 (n=29)	>44 (n=95)	
Age in years, mean (SD)	48 (16)	60 (13)	62 (14)	<0.001
Female, n(%)	13 (76)	22 (76)	58 (61)	0.21
Symptom duration in weeks, median (IQR)	26 (8-41)	12 (4-25)	12 (5-22)	0.13
(Sub)acute symptom onset, n(%)	3 (20)	7 (26)	46 (52)	0.01
66-SJC, median (IQR)	3 (2-7)	6 (3-13)	9 (3-13)	0.08
68-TJC, median (IQR)	12 (9-19)	11 (5-21)	9 (4-17)	0.23
RF positivity, n(%)	7 (41)	9 (31)	33 (35)	0.78
ESR (mm/h), median (IQR)	9 (4-14)	14 (6-33)	33 (19-48)	<0.001
CRP (mg/L), median (IQR)	3 (3-3)	3 (3-4)	22 (11-44)	<0.001

Characteristics of ACPA-negative RA patients with low, moderate or high MBDA score were compared with one-way ANOVA, chi-square test and Kruskal-Wallis test, as appropriate. ACPA, anti-citrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; MBDA, multi-biomarker disease activity; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; SJC, 66-swollen joint count; (sub)acute symptom onset, prompt onset of symptoms (<1 week); symptom duration, time between symptom onset and inclusion in cohort; TJC, 68-tender joint count.

Discussion

This is the first study showing that ACPA-negative RA patients with a high likelihood of achieving sustained DMARD-free remission during follow-up were identifiable at baseline by a combination of serological markers. This association with sustained DMARD-free remission was independent of clinical baseline

characteristics. Furthermore, the ACPA-negative subgroup with a high likelihood of achieving sustained DMARD-free remission showed some differences in clinical characteristics as they were older (mean ≥60 years) and more often had a (sub) acute symptom onset. Together, this suggests that a combination of serological biomarkers is helpful in identifying subgroups of ACPA-negative RA patients at disease presentation that differ in baseline characteristics and in their ability to maintain clinical remission after DMARD withdrawal.

Based on differences in genetic and environmental risk factors and in outcome, it is generally accepted that ACPA-positive and ACPA-negative RA are different RA subsets. In the past, we attempted to distinguish subgroups within the group of ACPA-negative RA patients based on only clinical characteristics at disease onset; this did not result in clinically distinguishable subgroups.[24] The current data suggest that a subdivision is possible with serological markers and that, starting from this subdivision, the identified subgroups had some slight differences in clinical characteristics as ACPA-negative RA patients with moderate or high serologic scores at disease onset were older, had more often a (sub)acute onset of symptoms and appeared to have greater inflammatory burden (reflected by higher levels of inflammatory proteins and a tendency towards more swollen joints). Thirty-eight percent of these patients were able to permanently stop DMARDs after a relatively short period of treatment, since DMARD-free remission was achieved after a median disease duration of 2.9 years, which means that DMARDs were stopped after median 1.9 years. Thus, the identified subgroup of ACPA-negative patients was older at disease onset and had more often a prompt onset of symptoms with more severe inflammation but a relative short-term necessity of DMARD treatment. Further studies are needed to confirm these findings.

It is unlikely that ACPA-negative RA patients were misclassified as having RA because patients that during the first year of follow-up were diagnosed with conditions other than RA (e.g. inflammatory osteoarthritis and reactive arthritis) were not included in this study. Also, patients that achieved spontaneous remission, i.e. without the use of DMARDs, were excluded. Patients studied here had a clinical diagnosis of RA and fulfilled classification criteria. In the current taxonomy, these patients are called RA patients. However, our data support the notion that subgroups can be identified within ACPA-negative RA.

A study of established RA patients with a median disease duration of 5 years, who were in sustained remission, showed that high MBDA scores during DMARD

treatment and prior to treatment reduction were associated with increased risk of relapses in patients who reduced, and in some cases, stopped, all their DMARD treatments.[25] This might be reflective of subclinical disease activity despite treatment and is conceptually very different from our data. In this study, the MBDA score was used to monitor disease activity, the aim for which the score was derived. In our data, we had a different aim for which measurements were performed in RA patients with very short symptom duration and before any DMARDs were initiated.

High MBDA scores have been associated with radiographic progression in several studies (although most did adjust but not stratify for ACPA).[11-13,26] In our study, performed at disease presentation, high MBDA scores strongly associated with a favourable outcome in ACPA-negative RA. This contrasts with the previous findings, but measurements in these studies were done in patients with a disease duration of several years and the studied outcomes were also different.

Our study was focused on achieving sustained DMARD-free remission. Within the group of ACPA-negative RA patients, patients with low MBDA score infrequently achieved this favourable outcome. Numerically, this group was relatively small (12% of ACPA-negative RA patients). Furthermore, this group resembled the ACPA-positive group of RA patients that also infrequently achieved DMARD-free remission. This ACPA-negative subgroup may be interesting for studies on (novel) autoantibody reactivities, as it is speculated that a 'serological gap' exists, meaning that part of ACPA-negative patients harbour unmeasured autoantibodies.[27] Moreover, our data revealed that sustained DMARD-free remission is a feasible outcome in about half of the ACPA-negative patients with moderate or high MBDA score.

A limitation is that although rheumatologists at our outpatient clinic are encouraged to try to taper and stop DMARDs in case of DAS remission, patients and rheumatologists were not forced to stop DMARDs if this was felt inappropriate and we did not record how often DMARD tapering was not done despite the presence of DAS remission and the absence of swollen joints. Consequently, the proportion of patients able to achieve sustained DMARD-free remission might be underestimated. It is particularly conceivable that either physicians or patients were reluctant with lowering or stopping medication in the presence of a positive ACPA test.

Another limitation is that the follow-up duration of some patients might have been insufficient to detect flares occurring years after the absence of synovitis, as this may occur after discharge from the outpatient clinic. For this study, patients needed to be in sustained DMARD-free remission for at least 1 year and patients were instructed to return to the outpatient clinic when symptoms recurred, an instruction that is facilitated by the presence of early arthritis recognition clinics and the fact that we are the only referral center in the region.[28] A final limitation is that the number of seronegative patients with low MBDA score was relatively small and therefore (multivariable) analyses within the ACPA-negative subgroup were of limited power resulting in wide confidence intervals of estimated hazard ratios. In addition, resampling methods to show robustness of the data were not performed. Therefore, validation of our results in another early RA cohort is needed.

Remission in this study was defined as the persistent absence of synovitis after DMARD cessation and thus was physician centred. Since synovitis needed to be persistently absent over time, this outcome is different from frequently used remission definitions that are used on single time points. Importantly, we have shown that patients who achieve sustained DMARD-free remission have normalization of functional status and of patient-reported outcomes, underlining that it is the best possible long-term outcome.[1]

The MBDA test comprised of serum levels of 12 proteins which were also evaluated separately. Of the different markers, CRP, SAA and MMP-3 were associated with achieving sustained DMARD-free remission. SAA is a protein linked to the acute phase response and is a sensitive indicator of RA disease activity.[29,30] MMP-3 is a proteinase considered to contribute to cartilage degradation in RA. Its levels have been associated with radiographic progression and also with disease activity and inflammation.[31-36] As the MBDA score was not designed to assess which patients might achieve DMARD-free remission, it is presumable that proteins other than the 12 that were studied here are also differently expressed in subgroups of ACPA-negative RA. Further studies are needed to better characterize this subgroup serologically. Additionally, biologic studies are needed to identify pathways that are relevant for the development of this subgroup of RA patients.

Conclusions

In conclusion, ACPA-negative RA patients who achieved sustained DMARD-free remission during follow-up were characterized by differences in protein expression at disease presentation. This is the first evidence that ACPA-negative RA can be subdivided at disease onset in clinically relevant subgroups with differences in the likelihood of achieving and maintaining clinical remission after treatment withdrawal.

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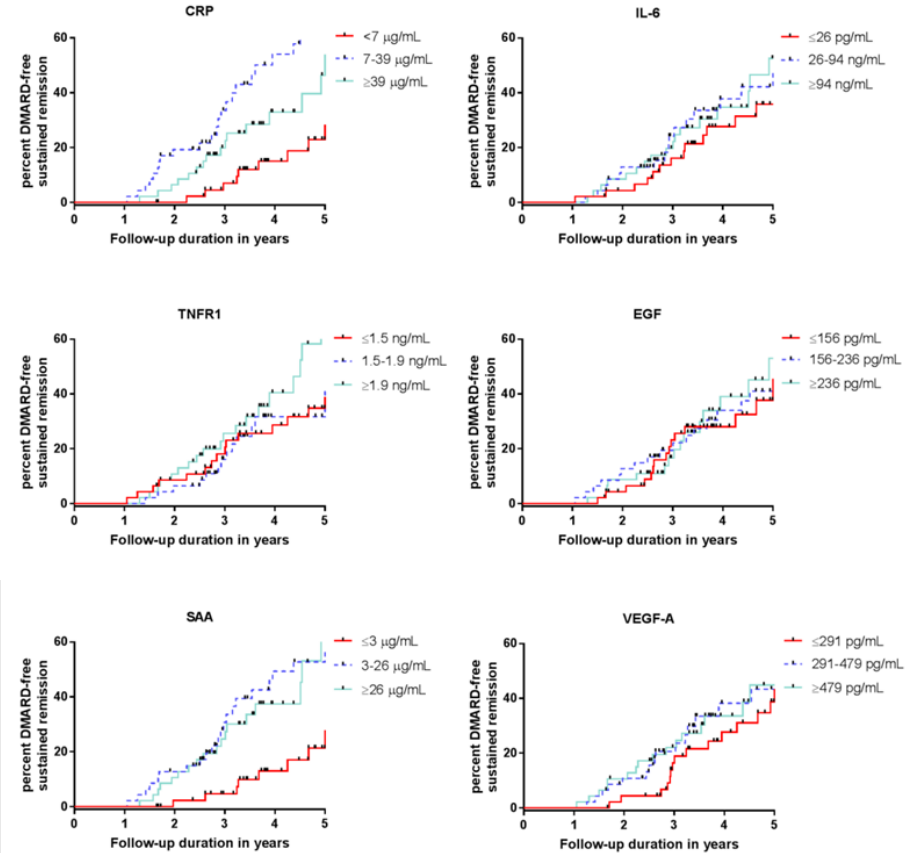
Supplementary material

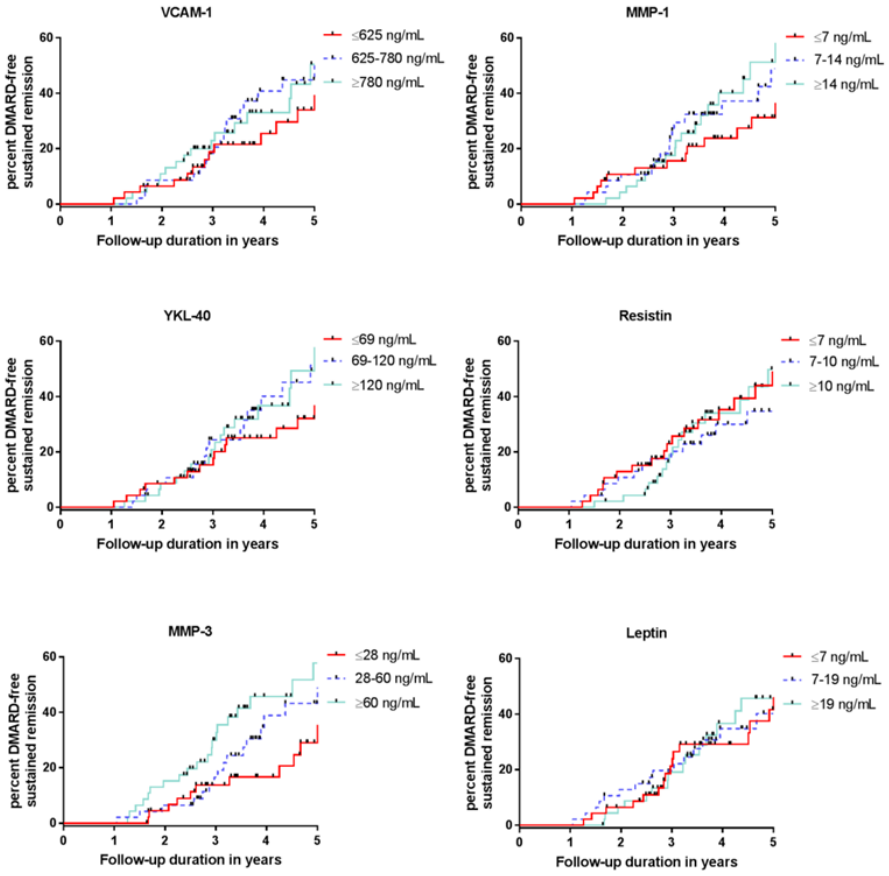
Supplementary table 10.1 Overview of medication used by all RA patients and by the subgroups of ACPA-positive and ACPA-negative RA patients during the total follow-up duration

	All RA patients (n=299)	Subgroup of ACPA-positive RA patients (n=158)	Subgroup of ACPA-negative RA patients (n=141)
Methotrexate, n(%)	271 (91)	152 (96)	119 (84)
Other conventional DMARDs, n(%)	254 (85)	140 (89)	114 (81)
Systemic glucocorticoids, n(%)	228 (76)	127 (80)	101 (72)
Sulfasalazine, n(%)	102 (34)	61 (39)	41 (29)
Hydroxychloroquine, n(%)	131 (44)	88 (56)	43 (30)
Leflunomide, n(%)	56 (19)	46 (29)	10 (7)
Azathioprine, n(%)	6 (2)	3 (2)	3 (2)
Biological DMARD, n(%)	60 (20)	48 (30)	12 (9)
TNF-inhibitor, n(%)	48 (16)	39 (25)	9 (6)
Rituximab, n(%)	3 (1)	2 (1)	1 (0.7)
Abatacept, n(%)	5 (2)	5 (3)	0 (0)
Tocilizumab, n(%)	16 (5)	14 (9)	2 (1)
Omalizumab, n(%)	5 (2)	5 (3)	0 (0)
Baricitinib, n(%)	1 (0.3)	1 (0.6)	0 (0)

Numbers indicate the number of patients that used the indicated medication at any time during follow-up. The number of patients using combination therapy, or of patients using several medications during follow-up is not specified here. ACPA, anti-citrullinated protein antibodies; DMARD, disease-modifying antirheumatic drug; RA, rheumatoid arthritis; TNF, tumor necrosis factor.



Supplementary figure 10.1 Kaplan-Meier plots showing achievement of sustained DMARD-free remission by ACPA-negative RA patients (n=141) grouped by tertiles of 12 serum biomarkers measured at disease presentation



Supplementary figure 10.1 continued

Higher CRP, SAA and MMP-3 levels were associated with achieving sustained DMARD-free remission. The other biomarkers were not associated with remission. Patients were categorized into tertiles based on the biomarker levels to create three groups of equal size. Vertical lines indicate that a patient is censored. CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; EGF, epidermal growth factor; IL-6, interleukin 6; MBDA, multi-biomarker disease activity; MMP-1, matrix metalloproteinase 1; MMP-3, matrix metalloproteinase 3; RA, rheumatoid arthritis; SAA, serum amyloid A; TNFR1, tumor necrosis factor receptor superfamily member 1A; VCAM-1, vascular cell adhesion molecule 1; VEGF-A, vascular endothelial growth factor A; YKL-40, human cartilage glycoprotein 39.

11

Summary and discussion

In this thesis, we aimed to get more insight into the earliest phases of rheumatoid arthritis (RA). It is clear that early treatment of RA patients is important to prevent joint damage progression and functional disability and to increase the chance of achieving remission. Although much research has focused on identifying RA patients as early as possible, risk stratification remains difficult. In **Part I** of this thesis we focused on the early recognition of RA patients. Different disease phases were studied. First, current knowledge about disease progression in patients with CSA (clinically suspect arthralgia) is explicated. Then, the phase of undifferentiated arthritis (UA) was studied in which the added value of different tools (antibodies, magnetic resonance imaging (MRI)) to early identification of RA patients was determined. In **Part II** the association between clinical and imaging features and the autoantibody response was investigated to get more insight into the mechanisms underlying RA.

Besides the fact that it is difficult to identify patients with imminent RA, it is also challenging to differentiate RA patients who will suffer from severe progression of their disease from patients who will have a slowly progressive disease. RA is considered to be a heterogeneous disease with the most common subdivision into anti-citrullinated protein antibodies (ACPA)-positive and ACPA-negative RA. In general, ACPA-positive RA patients have a worse prognosis with more erosive progression and lower chances of achieving a sustained disease-modifying antirheumatic drug (DMARD)-free status, which is the sustained absence of clinical synovitis after cessation of all DMARD therapy. However, not all ACPA-positive patients have a fast progressive disease, and there are also ACPA-positive patients who are able to achieve sustained DMARD-free remission. In **Part III** of this thesis the aim was to improve the understanding of mechanisms underlying sustained DMARD-free remission.

PART I
Early recognition of RA patients

RA is considered to consist of a preclinical phase in which patients first have no symptoms, but carry certain genetic and environmental risk factors and they can have different circulating autoantibodies.[1] Thereafter, symptoms of arthralgia might develop which eventually might progress to arthritis. The first phase in which patients with imminent RA can be identified is the phase of Clinically Suspect Arthralgia (CSA). Patients with CSA have a recent-onset arthralgia without

clinically detectable arthritis that is by the rheumatologist considered as at risk of progression to RA.[2]

In **Chapter 2**, a literature review was performed on the preclinical phase of RA. First of all, the relevance of adequate prediction making is discussed. Musculoskeletal symptoms are very prevalent and only 7% of the patients presenting with arthralgia at the rheumatologist, were identified as clinically suspect to progress to RA.[3] Furthermore, only 20% of these patients with CSA will develop arthritis, and only this subgroup might benefit from treatment to prevent disease progression.[4] Therefore, preventive trials in individuals with arthralgia should only include patients with a high risk of progression because otherwise the treatment effect is diluted and it might be falsely concluded that treatment in the phase of arthralgia has no beneficial value. To improve accurate risk prediction in this group of individuals, different biomarkers are needed. In the review, the predictive accuracy of autoantibodies, other serological markers and imaging markers for progression to arthritis in patients with arthralgia was assessed.

Although numerous predictors for progression from arthralgia to RA are studied, only the presence of ACPA is validated as independent risk factor in various studies.[4-8] Several major issues remain unexplored and therefore further research is warranted. One of the issues is that the majority of the studies included patients based on the presence of either ACPA or rheumatoid factor (RF), and therefore a seronegative patient group is frequently lacking. The predictive value of autoantibodies with autoantibody-negative RA patients as a reference group subsequently needs to be assessed. Other serological markers, such as C-reactive protein (CRP), were studied, but were shown to be of limited value. Again, the predictive value was mainly assessed in autoantibody-positive patients. Future studies, stratified for ACPA-positive and ACPA-negative arthralgia should be performed to determine the predictive value of serological markers in patients with ACPA-negative arthralgia. Since ACPA-positive and APCA-negative RA are considered separate disease entities, it is conceivable that predictors of disease progression are different between both patient groups.

Besides serological markers, the predictive accuracy of imaging markers detecting subclinical inflammation was reviewed in patients with arthralgia. Of the different imaging modalities, ultrasound (US) and MRI were most frequently studied. Unfortunately, performed studies differed in studied patient populations, joints that were assessed and the studied inflammatory features. Therefore,

the predictive value of US and MRI in patients with arthralgia for development of arthritis remains unclarified. Currently, it is debatable how an abnormal US or MRI should be defined. Previously, studies performed in healthy individuals showed that MRI-detected inflammation in metacarpophalangeal (MCP), wrist and metatarsophalangeal (MTP) joints is frequently observed and consideration of these findings when defining an abnormal MRI leads to an increased predictive accuracy.[9,10] Therefore, it seems reasonable to define a threshold at which MRI features should be regarded as abnormal. Whether it is also important to consider US-detected inflammation as present in healthy individuals when defining an abnormal US should be clarified. In addition, it is unresolved what joint features and which joints are most predictive and whether there is an additional value of assessing MTP joints next to MCP joints. When it appears that imaging of the foot can be omitted than the scanning time can be reduced.

Finally, in most cohort studies where the predictive value of several biomarkers was investigated, heterogeneous patient populations were included, e.g. arthralgia, seropositive arthralgia and ACPA-positive persons with non-specific musculoskeletal symptoms. Previously, a EULAR task force has defined a combination of symptoms and signs that characterize patients at risk of developing RA to enable inclusion of homogeneous sets of patients in studies.[2] This definition might be used in future studies to enhance comparability between studies and thereby, assess the predictive accuracy of biomarkers in a homogeneous group of patients with arthralgia. Thus, the predictive accuracy of most studied predictors is limited and most predictors were not evaluated relative to each other. Furthermore, other biomarkers than the ones studied are probably needed to arrive at optimal risk prediction in patients with CSA.

Early identification of RA patients is important because early treatment initiation is associated with an improved disease outcome, both in autoantibody-positive and autoantibody-negative RA.[11-14] To facilitate the early identification of RA patients, the 2010 ACR/EULAR criteria have been developed, which indeed have shown to be more specific than the previously used 1987 ACR criteria.[15-17] However, it was undetermined if the 2010 criteria identify both ACPA-positive and ACPA-negative patients earlier in time; this was subject of the study performed in **Chapter 3**. Patients from two different early arthritis cohorts were studied. For this study, patients were selected who fulfilled the 1987 criteria after one year follow-up, but not at the time of inclusion. When using the 1987 criteria these patients were thus classified as RA with some delay. Of these patients it was determined whether they

already fulfilled the 2010 criteria at disease presentation, and thus were identified earlier when using the 2010 criteria. Of the autoantibody-positive patients, 92-93% was identified earlier with the 2010 criteria within the two different cohorts. However, of the autoantibody-negative patients only 25-51% was identified earlier. This clearly indicates that ACPA-negative RA patients are still frequently missed by the 2010 criteria. Partly, this can be explained by the high weight that is given to the presence of ACPA and RF in the 2010 criteria. Although the 2010 criteria are classification criteria and were not developed for diagnosis, in clinical practise they can sometimes be used as such. To also diagnose ACPA-negative RA patients early in the disease process other diagnostics are therefore required.

One of the options to improve early identification is to search for novel autoantibodies. ACPA and RF are the most well-known autoantibodies and are considered a hallmark of RA but recently other autoantibodies have been identified, such as anti-peptidylarginine deiminase antibodies, anti-acetylated antibodies and anti-carbamylated protein (anti-CarP) antibodies.[18-20] Nonetheless, the clinical additional value of these autoantibodies was undetermined as the majority of the studies performed thus far did not stratify for the presence of ACPA and RF. This is important, since novel autoantibodies should have additional value to ACPA and RF to become clinically useful. Therefore we studied the additional diagnostic value of novel autoantibodies in **Chapter 4**. As an example the additional value of anti-CarP antibodies in predicting progression to RA for patients with UA was studied. It appeared that the additional value was dependent on the different classification criteria that are used. For patients with UA according to the 1987 criteria, anti-CarP antibodies were associated with progression to RA, independent of ACPA and RF (OR 1.7 95% CI 1.2-2.4). After stratification for ACPA and RF, anti-CarP antibodies were only associated with progression to RA within the ACPA- and RF-negative patient group (OR 2.1 95% CI 1.3-3.7). For patients with UA according to the 2010 criteria, anti-CarP antibodies were not associated with RA when analyses were corrected for ACPA and RF (OR 0.8, 95% CI 0.3-2.1). This observation is probably due to the fact that autoantibodies are heavily weighted in the 2010 criteria. Autoantibodies frequently occur together, thus patients who are positive for the novel autoantibody, are probably also positive for ACPA and/or RF and therefore the additional value of novel autoantibodies is limited. Perhaps other biomarkers such as other immunological markers present in the serum, or imaging markers might contribute to the early identification of RA. Finally, it is important to mention that this finding does not suggest that novel autoantibodies have no pathogenic role in RA; this is a completely different question and is subject

of future studies.

Since the additional value of novel autoantibodies appeared to be limited in the early identification of RA patients, it was studied whether imaging markers contribute to earlier identification of RA. To this end, the presence of MRI-detected erosions was studied. The use of MRI to detect damage at an earlier time point in RA than conventional radiographs is recommended.[21] It is already clear that MRI is more sensitive than radiography in detecting erosions.[22-29] However, it was undetermined whether the earlier identification of MRI-detected erosions contributes to the earlier identification of RA patients. This was studied in **Chapter 5** and **Chapter 6**. First, in **Chapter 5** the specificity of MRI-detected erosions for RA was determined. This was needed as MRI-detected erosions are also frequently observed in healthy individuals and in patients with other forms of arthritis.[9,30-34] Several characteristics of erosions present in MCP and MTP joints were compared between early RA patients, patients with other arthritides and symptom-free controls. This study revealed that total erosion scores, which are a combination of number and size of erosions, were comparable between the three groups. A few erosion characteristics were identified that were specific for RA when compared to symptom-free controls, which were grade ≥2 erosions, meaning that >20% of the bone was eroded, erosions in MTP5, erosions in MTP1 in patients aged <40 years at time of diagnosis and erosions with local inflammation in patients aged <60 years.

When RA patients were compared with patients with other arthritides instead of symptom-free controls, erosions combined with inflammation were not specific for RA anymore since these were also frequently observed in patients with other arthritides. Grade ≥2 erosions, erosions in MTP5 and erosions in MTP1 in patients aged <40 years remained specific for RA but these were present in a minority (21%) of RA patients.

The specificity of MRI-detected erosions was determined in patients who already received a clinical diagnosis. A subsequently and clinically relevant question is whether the identified RA-specific MRI-detected erosions are valuable in predicting progression in patients with UA, because they are at risk of developing RA and must be identified as early as possible. This was studied in **Chapter 6**. Besides development of RA, also the start of DMARDs within the first year of follow-up was studied, since autoantibody-negative RA patients require involvement of >10 joints to fulfil the 2010 classification criteria and because progression to RA might be hampered by DMARD treatment. 45% (128/286) of the UA patients developed

the outcome (2010-RA and/or DMARD start) within the first year. The previously identified RA-specific erosions were present in only 7% of the 2010-UA patients and were not associated with development of RA (OR 0.6, 95% CI 0.2-1.5, PPV 33%). Together these data demonstrate that although MRI is very sensitive in the detection of erosions, the value of MRI-detected erosions in the diagnostic process should not be overestimated.

PART II
Clinical and imaging features and the ACPA response

In this part, the association between clinical and imaging features and the autoantibody response was investigated to get more insight into the mechanisms underlying RA. In clinical practice conventional radiography is the most common used imaging modality but in research MRI and US are increasingly performed because with these modalities also inflammatory soft tissue changes can be visualized. A unique feature of MRI is the capability to detect bone marrow edema (BME). In RA patients these BME lesions consist of inflammatory cell infiltrates.[35-37] Several studies have shown that BME is a strong predictor of erosive progression.[38-46] Besides BME, also the presence of ACPA is strongly associated with erosive progression.[47-55] The association between ACPA and other autoantibodies, and BME had not been extensively studied before and was subject of **Chapter 7**. Intriguingly, we observed that the presence of ACPA alone was not associated with BME. However, the combined presence of ACPA with RF and/or anti-CarP antibodies was associated with more BME, suggesting an interactive effect between the different autoantibody systems.

Several studies have evaluated the interaction between ACPA and RF. Using in vitro assays in which macrophages were incubated with ACPA immune complexes in the presence or absence of monoclonal RF IgM, it was observed that the combined presence of ACPA and RF induced a higher production of pro-inflammatory cytokines by macrophages than the presence of ACPA alone.[56,57] Another study showed that the interaction between ACPA and citrullinated peptide targets was enhanced by the presence of RF, suggesting that the pathogenic effect of the combined presence of ACPA and RF might be explained by crosslinking immune complexes and thereby forming higher avidity immune complexes.[58] Surprisingly, the binding of RF to ACPA IgGs was similar to that of non-ACPA IgGs and RF binding was independent on the galactose content of the IgG constant domain, however it

was suggested that RF-ACPA IgG complexes may still preferentially be formed over RF-non-ACPA IgG complexes due to the abundance of ACPA.[59] Further fundamental studies should be performed to get more insight into the interaction between ACPA and RF.

In **Chapter 8**, the association between clinical characteristics and the autoantibody response (ACPA, RF and anti-CarP antibodies) was investigated in five different early arthritis cohorts. This revealed that at older age of disease onset, patients are more frequently ACPA-negative. In addition, several clinical parameters at disease onset in RA patients were different in patients with an older age at disease onset: patients were more often male, did not smoke, had higher acute phase reactants and had more often a (sub)acute onset of their symptoms. These data suggest that part of the ACPA-positive and ACPA-negative RA patients diagnosed at older age are comparable to patients of younger age, but there might also be a distinct subgroup of ACPA-negative patients preferentially presenting at older age with slight differences in clinical presentation and probably in underlying pathogenic mechanism. All patients in this study were diagnosed with RA which makes phenotypic misclassification of this ACPA-negative patient group very unlikely.

In a recent study, it was observed that part of the RA patients diagnosed at older age were initially diagnosed with polymyalgia rheumatica (PMR).[60] Male patients with PMR in this study had a higher risk to develop RA than female patients which is contradictory to RA development at younger age where the risk of RA is much higher for female patients. Interestingly, although the presence of ACPA was associated with progression to RA in patients with PMR, the majority of these patients were ACPA-negative. Although this study had a different design than our study, these data might also point towards a subgroup of RA patients at older age with some differences in clinical characteristics and outcome.

Together these data support the hypothesis that ACPA-negative RA at older age of onset has a different pathogenesis than ACPA-negative RA at younger age. A next step is to further validate this observation in other cohort studies. In addition, fundamental studies are required to evaluate whether the pathogenesis of ACPA-negative RA at older age is indeed different from that of younger age.

PART III
Resolution of rheumatoid arthritis

In part III, the focus was on the long-term outcome of RA patients. While traditionally joint damage was the most important outcome, this has become less relevant since damage can be prevented with current treatment strategies. Therefore, other long-term disease outcomes will become more important. One of these outcomes is the achievement of sustained DMARD-free remission, which is defined as the absence of clinical synovitis for at least one year that is sustained during the complete follow-up. Sustained DMARD-free remission is an increasingly achievable outcome and can be considered as the closest proxy of cure of RA.[61] The studies performed in part III of this thesis aimed to improve the understanding of mechanisms underlying a sustained DMARD-free status.

Recently, it was suggested that patients who are in immunological remission, defined as the disappearance of ACPA and RF, have the highest likelihood of achieving sustained DMARD-free remission.[62] However, this was all hypothesis based and therefore the association between ACPA and RF seroreversion and achievement of sustained DMARD-free remission was studied in **Chapter 9**. Of the anti-CCP2 IgG positive RA patients who achieved sustained DMARD-free remission, 12.8% had seroreverted when remission was achieved. However, in RA patients who had recurrence of synovitis after initially being in DMARD-free remission and in RA patients with persistent disease, seroreversion was observed in 8.3% and 5.7%, respectively, which was not significantly different from the patients who achieved sustained DMARD-free remission. Similar results were obtained for RF IgM.

The ACPA immune response has been shown to consist of various isotypes, which differ in their ability to mediate effector mechanisms. A typical immune response is characterized by the emergence of IgM antibodies after first antigen exposure, followed by the presence of IgG antibodies. After repeated antigen exposure, there is an increase in IgG antibodies while the IgM response disappears or lowers as compared to the primary response. In RA, it was shown that indeed IgM anti-CCP antibodies are present early in the disease course, however also after several years of follow-up, IgM antibodies remained present in the majority of patients who were positive for anti-CCP IgM at disease presentation, suggesting that there is continuous reactivation of the anti-CCP response.[63] We hypothesized that if dampening of the ongoing anti-CCP immune response is underlying the extinguishment of RA, anti-

CCP IgM antibodies would disappear. Therefore, in addition to anti-CCP2 IgG, also seroreversion rates for anti-CCP2 IgM were determined. However, patients who achieved sustained DMARD-free remission did not serorevert more frequently than patients who did not achieve remission, suggesting that the ACPA immune response is not underlying the maintained resolution of disease or that other characteristics of the ACPA response should be investigated.

As regards characteristics of the ACPA response, it is known that ACPA level is highly associated with the ACPA fine specificity repertoire and the number of ACPA isotypes, and therefore we anticipated that these characteristics were also not different between patients who did and did not achieve sustained DMARD-free remission.[64] Further studies are needed to elucidate whether these ACPA characteristics indeed remain unchanged as well. Another characteristic of the ACPA response which might be relevant to study with this respect is glycosylation of the constant (Fc) and variable (Fab) domain of ACPA IgG. Glycosylation is a reaction in which carbohydrates are attached to other molecules, in this case, autoantibodies. Previously, it was shown that ACPA IgG in RA patients have a changed Fc glycosylation pattern with reduced galactosylation and sialylation compared to that of total serum IgG.[65] This might have a proinflammatory effect by facilitating the formation of immune complexes and favoring the binding of IgG to activating FcγRs.[66] In addition, the Fab fragment of ACPA IgG is shown to be extensively glycosylated which might affect several antibody properties and B cell survival.[67-69] Thus, both changes in Fc and Fab glycans can have considerable impact on effector mechanisms of the autoimmune response and therefore it would be interesting to investigate whether the glycosylation profile of ACPA of patients who are in a sustained DMARD-free status has normalized by comparing it with that of non-ACPA IgG.

Another possibility is that not characteristics of ACPA itself, but of the ACPA producing B cells are associated with achievement of sustained DMARD-free remission. Recently, a technology was developed to identify and isolate citrullinated antigen-specific B cells from peripheral blood of RA patients.[70] This technology was used to compare the phenotype of citrullinated antigen specific B cells with tetanus-toxoid specific B cells of the same patient. The majority of the isolated cells had a memory phenotype and it appeared that citrullinated antigen specific B cells overexpress co-stimulatory molecules and proliferation markers, indicating the presence of an active immune response.[71] It would be very exciting to measure these markers (CD80, CD68, HLA-DR and Ki67) in patients who are in a sustained

DMARD-free status.

Besides B cells, T cells could be the driving force underlying disease persistence. Several changes in the composition and characteristics of the T cell compartment have been described in patients in remission under DMARD treatment.[72,73] Thus far, no studies have looked into the T cell compartment of patients in sustained DMARD-free remission. Overall, future studies are needed to define which immunological marker is the best reflection of disease persistence, and thus to determine the optimal definition of immunological remission.

Although sustained DMARD-free remission is an increasingly achievable outcome, still only a minority of RA patients is able to achieve this outcome. In **Chapter 10** the aim was to get more insight into this subgroup of patients. Clinical characteristics and autoantibodies alone are insufficient to assess which patients have a favorable outcome of their disease. We hypothesized that other serological markers than autoantibodies might contribute to the differentiation of subgroups of patients who have different chances of achieving sustained DMARD-free remission during follow-up. To this end, twelve different biomarkers (CRP, IL-6, SAA, TNFR1, EGF, VEGF-A, VCAM-1, MMP-1, MMP-3, YKL-40, resistin and leptin) were measured in serum samples of RA patients, collected at disease presentation. We started by measuring these biomarkers because these were already selected from a larger pool of markers by a company and were already shown to be associated with RA disease activity. 299 RA patients were followed for median 4.3 years of which 20% achieved sustained DMARD-free remission. Among ACPA-positive RA patients, biomarker scores were not associated with achieving sustained DMARD-free remission, while among ACPA-negative RA patients, moderate or high scores associated strongly with DMARD-free remission (moderate vs. low HR 9.4, 95% CI 1.2-72.9, high vs. low HR 9.7 95% CI 1.3-71.1). This association was independent of clinical characteristics (high vs. low HR 8.2 95% CI 1.1-61.8), showing the additive value of the serological markers. To get more insight into the markers driving this finding, the association with sustained DMARD-free remission was evaluated for each biomarker separately, revealing the largest associations for CRP, SAA and MMP-3 in ACPA-negative patients. This was the first time that ACPA-negative RA patients could be divided into subgroups with differences in long-term outcome using a protein profile.

Intriguingly, ACPA-negative RA patients with higher serological scores, indicating high disease activity at baseline, presenting with a (sub)acute onset of symptoms

and with more swollen joints, had the highest chance of achieving sustained DMARD-free remission during follow-up. Previously these clinical characteristics were insufficient in subdividing ACPA-negative RA, but here we show that when combined with serological markers, differentiation of ACPA-negative patients was improved.[74] Although our findings are promising and may open possibilities for personalized treatment aiming at disease resolution in ACPA-negative RA, results should be validated in another early arthritis cohort. Furthermore, in the future certainly also other biomarkers should be investigated to further characterize this ACPA-negative subgroup. Eventually this will contribute to the identification of pathways that are relevant for the development of this subgroup of RA patients.

Final considerations

Since it has become clear that early treatment of RA patients is needed to improve disease outcome, research has shifted towards identification of patients as early as possible. Part of the studies described in this thesis focused on the early identification of RA. Overall it can be concluded that more research is needed to arrive at adequate risk prediction for progression to RA in both patients with CSA and in patients with UA. One of the difficulties is that RA is a heterogeneous disease probably consisting of separate disease subsets besides ACPA-positive and ACPA-negative RA. ACPA-negative RA in particular is considered to be a heterogeneous group and especially for the ACPA-negative group more predictors are needed. Adequate risk stratification is crucial, especially for interpretation of results from trials in which at-risk patients are treated to prevent progression to RA. When patients with low risk of progression are included in these trials it may be falsely concluded that preventive treatment has no beneficial effect. Therefore, further research in observational longitudinal studies should be performed and probably a combination of different markers (clinical, serological and imaging) is needed.

Sustained DMARD-free remission is a very intriguing long-term outcome as it is the best approximation of cure of RA, nonetheless, it is infrequently studied. Partly, this can be explained by differences in treatment strategies in different countries. In the LUMC it is common practice to try to taper and stop DMARD therapy when patients have persistent low disease activity, while in outpatient clinics in other countries rheumatologists might be more reluctant with stopping DMARD therapy. This difference in treatment strategy might be due to the fact that currently it is unknown which RA patients can safely and successfully stop their

medication without recurrence of arthritis.[62,75] Another important notion is that sustained DMARD-free remission is a rather subjective outcome. When patients have a persistent low disease activity, the rheumatologist and the patient together decide whether or not to stop treatment. Presumably, rheumatologists are more reluctant to stop medication when patients are ACPA-positive. Furthermore, it is unknown what happens with patients after achievement of sustained DMARD-free remission. In the LUMC, RA patients who are in sustained DMARD-free remission are referred to their general practitioner and are instructed to return when symptoms recur. In addition, there are early access clinics to promote early access to rheumatologic care.[76] Therefore it can be presumed that patients who do not return to the outpatient clinic remain in a sustained DMARD-free status.

Biological mechanisms underlying achievement of a sustained DMARD-free status remain unknown. Disappearance of autoantibodies was not associated with sustained DMARD-free remission, suggesting that absence of autoantibodies is not important for the maintained resolution of disease. Future studies should reveal whether changes in other immunological markers (e.g. B cell or T cell characteristics) are relevant. Besides understanding the underlying mechanisms of extinguishment of disease it is also important to identify patients who have the highest chance of a favorable outcome and who profit the most from DMARD cessation because DMARDs are expensive and have side-effects. Currently, absence of ACPA is the most important predictor for achievement of sustained DMARD-free remission, but more predictors are needed. One of the difficulties when studying the value of serological biomarkers in predicting sustained DMARD-free remission is that the moment when medication is attempted to stop is rather subjective. Therefore the moment of stopping medication does not necessarily indicate similar disease states between different patients. Some patients might have cure of their disease for a long period (although they use medication), while other patients have just recently become in disease remission. Although these patients might be considered as being in the same disease state when they are in sustained DMARD-free remission, biomarker profiles might be completely different. Another challenge is to find biomarkers that not merely reflect and coincide with disease activity, as it was observed for example for IP-10, but change before disease has been extinguished.[77] Results from observational cohort studies and randomized controlled trials should clarify in which patient group DMARD tapering and stopping is worthwhile to consider. Eventually, a combination of biomarkers is probably needed, as is the case for early identification of RA.

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12

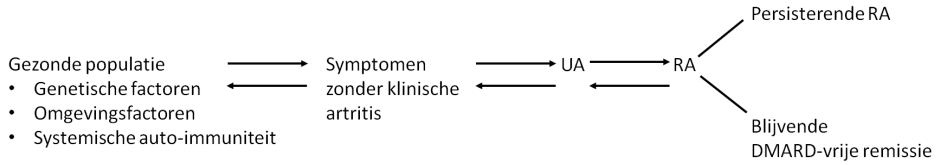
Nederlandse samenvatting

Reumatoïde artritis (RA) is een chronische ziekte die vooral wordt gekenmerkt door gewrichtsontsteking (artritis) van de kleine hand- en voetgewrichten. Met een prevalentie van 0.5-1% is het de meest voorkomende reumatische ziekte in de westerse bevolking. De ziekte komt drie keer vaker voor bij vrouwen dan bij mannen en de incidentie neemt toe met de leeftijd.

De gewrichtsontstekingen gaan gepaard met pijn, zwelling en ochtendstijfheid wat zonder adequate behandeling kan leiden tot destructie van de gewrichten. Naast ontsteking van de gewrichten, kunnen ook ontstekingen van de inwendige organen optreden, zoals de longen, hart, nieren en bloedvaten. RA is een complexe, multifactoriële ziekte en wordt beschouwd als een auto-immuunziekte omdat bij ruim tweederde van de patiënten autoantistoffen in het bloed aanwezig zijn. De meest voorkomende autoantistoffen zijn reumafactor (RF) en antistoffen tegen gecitrullineerde eiwitten (ACPA). Met name ACPA zijn kenmerkend voor RA waarbij ACPA-positieve en ACPA-negatieve RA worden gezien als verschillende subsets van RA met verschillende onderliggende pathogenese en genetische risicofactoren. Daarnaast zijn zowel ACPA als RF geassocieerd met relevante uitkomstmaten zoals radiografische schade en het behalen van remissie.

De laatste decennia is de behandeling van RA sterk verbeterd door de ontwikkeling van nieuwe effectieve medicatie, waaronder 'disease-modifying antirheumatic drugs' (DMARDs) en 'biologicals'. Daarnaast heeft het vroeg starten van behandeling en het gebruik van ziekteactiviteit scores bijgedragen aan de verbeterde behandeling van RA. Omdat vroege behandeling belangrijk is om progressie van gewrichtsschade en functionele achteruitgang te voorkomen, richt veel onderzoek zich op het zo vroeg mogelijk identificeren van RA patiënten. De ontwikkeling van RA kan worden verdeeld in verschillende ziekte fasen. Voordat symptomen ontstaan, kunnen er al genetische risicofactoren of omgevingsrisicofactoren aanwezig zijn. Daarnaast kunnen autoantistoffen ook al jaren voor het ontstaan van RA aanwezig zijn. In de fase van 'clinically suspect arthralgia' (CSA) hebben patiënten recent ontstane gewrichtsklachten die volgens klinisch expertise van de reumatoloog verdacht zijn voor het ontwikkelen van RA. In deze fase hebben patiënten wel al symptomen maar heeft er zich nog geen klinische artritis ontwikkeld. Maar een klein deel van deze CSA patiënten ontwikkelt artritis tijdens follow-up. Onderzoek in deze groep patiënten is van belang om uiteindelijk beter te kunnen voorspellen welke CSA patiënten progressie naar artritis zullen hebben, en mogelijk baat hebben bij vroege behandeling, en bij welke patiënten behandeling beter achterwege kan worden gelaten omdat zij nooit artritis zullen

ontwikkelen. De fase waar klinische artritis kan worden vastgesteld is de fase van ongedifferentieerde artritis (UA). Van de patiënten met UA zal ongeveer de helft spontane remissie behalen, terwijl ongeveer een derde RA zal ontwikkelen. Van de patiënten met RA zal de meerderheid persisterende ziekte hebben. Echter voor een deel van de patiënten is het mogelijk om blijvende DMARD-vrije remissie te behalen. Dit houdt in dat na het staken van alle medicatie er geen klinische artritis meer wordt vastgesteld en er dus genezing van de ziekte lijkt te zijn ontstaan. Bovengenoemde ziektefasen zijn weergegeven in Figuur 12.1. De verschillende ziektefasen worden niet door alle patiënten doorlopen en volgen elkaar ook niet noodzakelijk in dezelfde volgorde op.



Figuur 12.1 Verschillende fasen van RA ontwikkeling

Identificatie van de patiënten met CSA met uiteindelijk progressie naar RA is een uitdaging omdat de meeste patiënten nooit RA zullen ontwikkelen. Een aantal factoren zijn geassocieerd met het ontwikkelen van RA in patiënten met CSA, waaronder de aanwezigheid van autoantistoffen en de aanwezigheid van subklinische ontsteking van de gewrichten, zoals vastgesteld op magnetic resonance imaging (MRI). Hoofdstuk 2 van dit proefschrift geeft een overzicht van de literatuur over de preklinische fase van RA. Hoewel er veel verschillende voorspellers voor progressie van artralgie naar RA zijn onderzocht, zijn alleen ACPA aangetoond als onafhankelijke risicofactor in meerdere studies. De toegevoegde waarde van andere markers in het bloed is nog niet aangetoond. Ook de waarde van echografie en MRI om gewrichtsontsteking in een vroege fase vast te stellen, is nog onduidelijk. Er is dus meer onderzoek nodig om tot adequate risicopredictie te komen binnen patiënten met CSA.

In de klinische praktijk wordt de diagnose RA gesteld door de reumatoloog op basis van een combinatie van klinische symptomen en bevindingen bij lichamelijk en aanvullend onderzoek. De diagnose RA is dus in feite gebaseerd op patroonherkenning en er is geen simpele diagnostische test om RA aan te tonen

wat resulteert in een heterogene groep van patiënten. Om de resultaten van studies met RA patiënten met elkaar te kunnen vergelijken, is het echter belangrijk om een relatief homogene groep van RA patiënten te hebben. Om dit te bereiken zijn in de loop der jaren verschillende classificatiecriteria ontwikkeld. In huidige studies worden zowel de 1987 ACR criteria als de 2010 ACR/EULAR criteria gebruikt. De 1987 criteria zijn ontwikkeld om de specificiteit te verhogen ten opzichte van de oudere criteria, dus om het onderscheid tussen patiënten met RA en patiënten met artritis door een andere ziekte te verbeteren. Een nadeel van de 1987 criteria is dat patiënten in de vroege fase van RA minder goed worden geïdentificeerd dan patiënten met gevorderde RA. Om de identificatie van patiënten in de vroege fase van RA te verbeteren, zijn de 2010 criteria ontwikkeld. Het grootste verschil tussen de 2010 en 1987 criteria is dat de 2010 criteria zijn gericht op kenmerken van vroege artritis die geassocieerd zijn met persisterende ziekte en met de ontwikkeling van schade. Daarom zijn de aanwezigheid van acute fase eiwitten (CRP en BSE) en ACPA toegevoegd aan de criteria. Kenmerken die passen bij gevorderde ziekte, zoals de aanwezigheid van reuma noduli en radiografische erosies werden niet meer in de 2010 criteria meegenomen. Meerdere studies hebben laten zien dat de 2010 criteria inderdaad beter zijn in het identificeren van vroege RA patiënten dan de 1987 criteria, dus de sensitiviteit is hoger. Dit gaat echter ten koste van een lagere specificiteit wat inhoudt dat er ook patiënten zijn die aan de 2010 criteria voldoen die geen RA hebben maar artritis door een andere ziekte. Het was tot nu toe echter nog onduidelijk of met de 2010 criteria zowel ACPA-positieve als ACPA-negatieve patiënten in een vroegere fase van de ziekte worden geïdentificeerd. Dit is onderzocht in hoofdstuk 3 van dit proefschrift. In deze studie werden patiënten van twee verschillende vroege artritis cohorten onderzocht. Patiënten die voldeden aan de 1987 criteria na een jaar follow-up, maar niet op het moment van inclusie, werden geselecteerd. Deze RA patiënten werden dus met de 1987 criteria niet op het moment van presentatie geïdentificeerd, maar pas later tijdens follow-up. Van deze patiënten is bekeken of zij al wel voldeden aan de 2010 criteria op het moment van presentatie met klachten. Indien dit het geval zou zijn dan zouden deze patiënten dus met de 2010 criteria eerder worden geclassificeerd als RA dan met de 1987 criteria. Van de autoantistof positieve RA patiënten werd 92-93% van de patiënten eerder geïdentificeerd met de 2010 criteria binnen de twee verschillende cohorten. Van de autoantistof negatieve RA patiënten werd echter maar 25-51% eerder geïdentificeerd. Dit laat duidelijk zien dat de 2010 criteria met name goed zijn in het zo vroeg mogelijk identificeren van autoantistof positieve patiënten maar dat autoantistof negatieve patiënten nog steeds frequent worden gemist. Dit kan deels worden verklaard doordat de aanwezigheid van ACPA en

RF zwaar worden gewogen in de 2010 criteria. Patiënten die deze autoantistoffen niet hebben, moeten veel meer aangedane gewrichten hebben om alsnog aan de 2010 criteria te kunnen voldoen. Deze bevinding is belangrijk aangezien het bekend is dat vroege behandeling, zowel van ACPA-positieve als ACPA-negatieve RA, belangrijk is om de ziekte uitkomst te verbeteren.

Ondanks dat de classificatie criteria zijn ontwikkeld voor wetenschappelijk onderzoek en niet voor het stellen van de diagnose RA, worden ze in de klinische praktijk soms wel zo gebruikt. Ongeveer een derde van de patiënten met RA zijn negatief voor zowel ACPA als RF. Om ook deze autoantistof negatieve patiënten eerder te diagnosticeren zijn andere diagnostische tools nodig. Huidig onderzoek richt zich onder andere op het ontdekken van andere, nieuwe autoantistoffen, om ook deze groep patiënten zo vroeg mogelijk te identificeren. In hoofdstuk 4 is gekeken naar de toegevoegde waarde van een van die nieuwe autoantistoffen, namelijk naar antistoffen tegen gecarbamyleerde eiwitten (anti-CarP antistoffen). Dit is onderzocht binnen patiënten met UA. Dit zijn patiënten met klinische artritis maar zij voldoen nog niet aan RA classificatiecriteria. Het bleek dat de toegevoegde waarde van anti-CarP antistoffen afhankelijk is van de classificatiecriteria die worden gebruikt. Binnen ACPA en RF negatieve UA patiënten volgens de 1987 criteria was de aanwezigheid van anti-CarP antistoffen geassocieerd met de ontwikkeling van RA. In deze groep steeg het risico op ontwikkeling van RA van 21% naar 35% wanneer anti-CarP antistoffen aanwezig waren. Echter wanneer RA werd gedefinieerd volgens de 2010 criteria was er geen toegevoegde waarde van anti-CarP antistoffen. Deze bevinding kan worden verklaard door het grote gewicht dat aan ACPA en RF wordt gegeven binnen de 2010 criteria. Als gevolg hiervan zijn de meeste UA patiënten ACPA en RF negatief. Het is bekend dat autoantistoffen vaak samen voorkomen waardoor patiënten die positief zijn voor ACPA en RF ook vaker positief zullen zijn voor een andere antistof; andersom zijn patiënten die negatief zijn voor ACPA en RF ook vaker negatief voor een andere antistof. Dit is belangrijk omdat nieuwe autoantistoffen alleen van klinisch toegevoegde waarde zullen zijn wanneer zij iets toevoegen aan ACPA en RF. Voor anti-CarP antistoffen lijkt de toegevoegde diagnostische waarde beperkt te zijn. Gezien het frequent samen voorkomen van autoantistoffen zal dit voor andere nieuwe autoantistoffen wellicht ook het geval zijn. Om de vroege identificatie van RA te verbeteren, kan daarom misschien beter gezocht worden naar andere markers, zoals immunologische markers die aanwezig zijn in het serum, of naar markers die te zien zijn met beeldvorming, zoals tekenen van ontsteking of aanwezigheid van erosies.

In hoofdstuk 5 en 6 is de aanwezigheid van erosies bestudeerd met behulp van MRI. Het gebruik van MRI wordt al aangeraden om schade eerder in het ziekteproces aan te tonen dan met conventionele röntgenfoto's. Het is echter nog onbekend of het vroeger aantonen van MRI-gedetecteerde erosies leidt tot eerdere identificatie van RA patiënten. In hoofdstuk 5 is onderzocht of MRI-gedetecteerde erosies in hand (MCP) en voet (MTP) gewrichten specifiek zijn voor RA of dat ze ook vaak worden gezien in gezonde mensen of patiënten met andere vormen van artritis. Hiervoor is gekeken naar verschillende erosie karakteristieken. Wanneer RA patiënten werden vergeleken met gezonde mensen waren een aantal erosie karakteristieken specifiek voor RA. Dit waren grote erosies (>20% van het bot aangedaan), erosies in MTP5, erosies in MTP1 in patiënten jonger dan 40 jaar op het moment van diagnose, en erosies met lokale ontsteking in hetzelfde bot in patiënten jonger dan 60 jaar. Erosies met lokale ontsteking waren niet meer specifiek voor RA wanneer RA patiënten werden vergeleken met patiënten met andere vormen van artritis in plaats van met gezonde mensen. Dit komt omdat erosies samen met ontsteking ook veel bij andere vormen van artritis wordt gezien. Grote erosies, erosies in MTP5 en erosies in MTP1 in patiënten jonger dan 40 jaar op het moment van diagnose bleven specifiek voor RA maar deze waren aanwezig in maar 21% van de RA patiënten. Een klinisch relevante vervolgvraag is of de aanwezigheid van RA specifieke erosies bijdraagt aan het voorspellen van het ontwikkelen van RA in patiënten met een verhoogd risico op RA. Dit is onderzocht in hoofdstuk 6. Hiertoe is gekeken binnen UA patiënten. Het bleek dat de eerder gevonden RA specifieke erosies maar aanwezig waren in 7% van de UA patiënten. Daarnaast was de aanwezigheid van deze erosies niet geassocieerd met het ontwikkelen van RA. Samenvattend, laat dit onderzoek zien dat MRI erg sensitief is in het vaststellen van erosies, maar dat de waarde van MRI-gedetecteerde erosies in het diagnostisch proces niet overschat moet worden aangezien erosies ook in gezonde mensen en patiënten met andere vormen van artritis aanwezig zijn.

Het is bekend dat de aanwezigheid van autoantistoffen is geassocieerd met het ontstaan van RA, echter de precieze rol van autoantistoffen in de ziekte is nog onbekend. Onderzoek beschreven in hoofdstuk 7 en 8 is verricht om hier meer inzicht in te krijgen. In hoofdstuk 7 is de relatie tussen inflammatie zoals gezien op MRI en de aanwezigheid van autoantistoffen onderzocht. Een unieke eigenschap van MRI is dat beenmergoedeem kan worden afgebeeld. Beenmerg oedeem laesies zijn afwijkingen die ontstaan door de vervanging van beenmerg vet door een ontstekingsinfiltraat. Verschillende studies hebben laten zien dat de aanwezigheid van beenmergoedeem een sterke voorspeller is voor het ontstaan van erosies

in het bot. Naast beenmergoedeem, zijn ACPA een sterke voorspeller voor het ontstaan van erosies. De associatie tussen ACPA en andere autoantistoffen, en het ontstaan van beenmergoedeem was nog niet bekend. Verrassend genoeg bleek uit ons onderzoek dat de aanwezigheid van ACPA alleen niet geassocieerd is met beenmergoedeem. Echter, de combinatie van ACPA met RF of anti-CarP antistoffen was wel geassocieerd met aanwezigheid van meer beenmergoedeem. Dit suggereert dat er een interactief effect is tussen de verschillende autoantistof systemen. Meer onderzoek zal moeten worden verricht om hier meer inzicht in te krijgen.

In hoofdstuk 8 is de associatie tussen klinische karakteristieken en de autoantistof respons onderzocht. Hieruit bleek dat patiënten die pas op oudere leeftijd RA krijgen, vaker ACPA-negatief zijn dan patiënten op jonge leeftijd. Daarnaast waren een aantal klinische parameters bij presentatie met RA verschillend tussen jonge en oude patiënten. Oudere RA patiënten waren vaker man, rookten niet, hadden meer acute fase eiwitten in het bloed en vaker een (sub)acute presentatie van hun symptomen. Deze data suggereren dat een deel van de ACPA-positieve en ACPA-negatieve patiënten die worden gediagnosticeerd op oudere leeftijd vergelijkbaar zijn met patiënten op jongere leeftijd, maar ook dat er mogelijk een ACPA-negatieve subgroep van patiënten bestaat op oudere leeftijd die een andere klinische presentatie heeft met mogelijk een andere onderliggend mechanisme van ontstaan dan ACPA-negatieve patiënten op jongere leeftijd. Verder fundamenteel onderzoek is nodig om te zien of dit inderdaad het geval is.

In het laatste deel van dit proefschrift is de lange termijn uitkomst van RA patiënten onderzocht. Hiertoe is het behalen van blijvende DMARD-vrije remissie bestudeerd. Maar een klein deel van de RA patiënten kan deze uitkomst behalen en biologische processen die ten grondslag liggen aan het uitdoven van de ziekte zijn nog onduidelijk. Het is gesuggereerd dat autoantistoffen de drijvende kracht zijn voor persisterende inflammatie in RA en dat daarom het verdwijnen van autoantistoffen mogelijk is geassocieerd met een grote kans op het behalen van blijvende DMARD-vrije remissie. Echter, de associatie tussen het verdwijnen van ACPA en RF en blijvende remissie is nog onvoldoende verduidelijkt en was daarom onderzocht in hoofdstuk 9. Het bleek dat van de ACPA-positieve RA patiënten die blijvende DMARD-vrije remissie haalden, 13% geen ACPA meer hadden op het moment dat zij in remissie waren. Van de ACPA-positieve patiënten met persisterend RA, waarbij het niet mogelijk was om medicatie te stoppen, werd 6% negatief voor ACPA tijdens follow-up. Deze percentages waren niet significant

verschillend. Vergelijkbare resultaten warden gezien voor RF. Dit suggereert dat de autoantistof respons niet ten grondslag ligt aan het behalen van blijvende DMARD-vrije remissie. Het sluit echter niet uit dat andere karakteristieken van de autoantistof respons een rol spelen.

Blijvende DMARD-vrije remissie wordt maar door een klein deel van de RA patiënten behaald. Klinische kenmerken en autoantistoffen alleen zijn onvoldoende om te voorspellen voor welke patiënten het mogelijk is om deze uitkomst te behalen. In hoofdstuk 10 is onderzocht of serologische markers, anders dan autoantistoffen, bijdragen aan het differentiëren van subgroepen binnen RA met een verschillende kans op het behalen van DMARD-vrije remissie tijdens follow-up. Twaalf verschillende biomarkers (CRP, IL-6, SAA, TNFR1, EGF, VEGF-A, VCAM-1, MMP-1, MMP-3, YKL-40, resistin and leptin) zijn gemeten in serum samples van RA patiënten op het moment van presentatie met RA. Onder ACPA-positieve patiënten was de combinatie van deze biomarkers niet geassocieerd met het behalen van blijvende DMARD-vrije remissie. Onder ACPA-negatieve patiënten daarentegen, waren de biomarkers wel geassocieerd met het behalen van remissie. Deze associatie was onafhankelijk van klinische kenmerken wat betekent dat er een toegevoegde waarde is van de gemeten biomarkers. Dit is de eerste keer dat de groep van ACPA-negatieve RA patiënten kan worden onderverdeeld in subgroepen met verschillen in lange termijn uitkomst. Het is opvallend dat ACPA-negatieve patiënten met hogere serologische scores, wijzend op hoge ziekte activiteit op moment van presentatie met RA, een grotere kans hadden om blijvende DMARD-vrije remissie te behalen. Resultaten van deze studie kunnen uiteindelijk bijdragen aan gepersonaliseerde behandeling van RA patiënten, echter eerst zal het onderzoek gevalideerd moeten worden in andere studies.

Conclusie

Om de ziekte uitkomst van RA patiënten te verbeteren, is het belangrijk om de diagnose zo vroeg mogelijk te stellen zodat behandeling vroeg in het ziekteproces gestart kan worden. Klinische kenmerken van de patiënt zijn hierbij belangrijk, maar bijvoorbeeld ook de aanwezigheid van autoantistoffen of van subklinische inflammatie zoals gezien met behulp van echo of MRI. Echter de combinatie van deze verschillende biomarkers voorspelt vooralsnog onvoldoende welke patiënten met gewrichtsklachten progressie van hun ziekte zullen hebben. Ook het voorspellen van de ziekte uitkomst blijft een uitdaging. Een van de moeilijkheden die hierbij

een rol speelt is dat RA een heterogene ziekte is die meest waarschijnlijk bestaat uit verschillende ziekte subsets, naast het onderscheid tussen ACPA-positieve en ACPA-negatieve RA.

De meest klinisch relevante uitkomst onder RA patiënten is het bereiken van blijvende DMARD-vrije remissie. Uit onderzoek beschreven in dit proefschrift blijkt dat een subgroep van ACPA-negatieve RA patiënten een grote kans heeft op het behalen van deze uitkomst. Onderliggende mechanismen voor het behalen DMARD-vrije remissie blijven vooralsnog onbekend; dit zal onderwerp zijn van toekomstige studies.

A

Curriculum Vitae
List of publications
Dankwoord

Curriculum Vitae

Debbie Maria Boeters werd geboren op 13 oktober 1989 in Naarden. In 2007 behaalde zij cum laude haar gymnasium diploma aan het Emmaus College te Rotterdam en begon zij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam. Haar keuzecoschap voltooide zij op de afdeling neurologie in het Maasstad ziekenhuis en haar oudste coschap op de afdeling interne geneeskunde in het IJsselland ziekenhuis. In 2014 behaalde zij het artsexamen.

Naast haar studie geneeskunde begon zij in 2010 met de tweejarige research master Infection and Immunity in Rotterdam. Voor haar research master heeft zij onderzoek gedaan op de afdeling longziekten in het Erasmus Medisch Centrum (EMC), naar de aanwezigheid en invloed van inflammatoire dendritische cellen en T-helper 2 cellen in het beenmerg van muizen met huisstofmijt-geïnduceerd astma. Daarnaast heeft zij op de afdeling reumatologie in samenwerking met de afdeling dermatologie in het EMC, onderzoek gedaan naar het effect van dimethylfumaraat op een Imiquimod-geïnduceerd psoriasis muismodel en op een pDC cellijn. In 2015 voltooide zij de research master.

Hierna startte zij met haar promotieonderzoek op de afdeling reumatologie in het Leids Universitair Medisch Centrum (LUMC) onder begeleiding van prof. dr. A.H.M. van der Helm-van Mil en prof. dr. T.W.J. Huizinga. Het onderzoek richtte zich op de vroege herkenning van patiënten met reumatoïde artritis en op het behalen van blijvende DMARD-vrije remissie, wat momenteel de beste klinische uitkomstmaat is.

In januari 2019 is zij gestart met haar opleiding tot reumatoloog in het LUMC. Momenteel volgt zij de vooropleiding interne geneeskunde in het Groene Hart Ziekenhuis te Gouda.

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Dankwoord

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