Clinically suspect arthralgia: unraveling the development of rheumatoid arthritis

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Clinically Suspect Arthralgia

Unraveling the Development of Rheumatoid Arthritis

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Clinically Suspect Arthralgia

Unraveling the Development of Rheumatoid Arthritis

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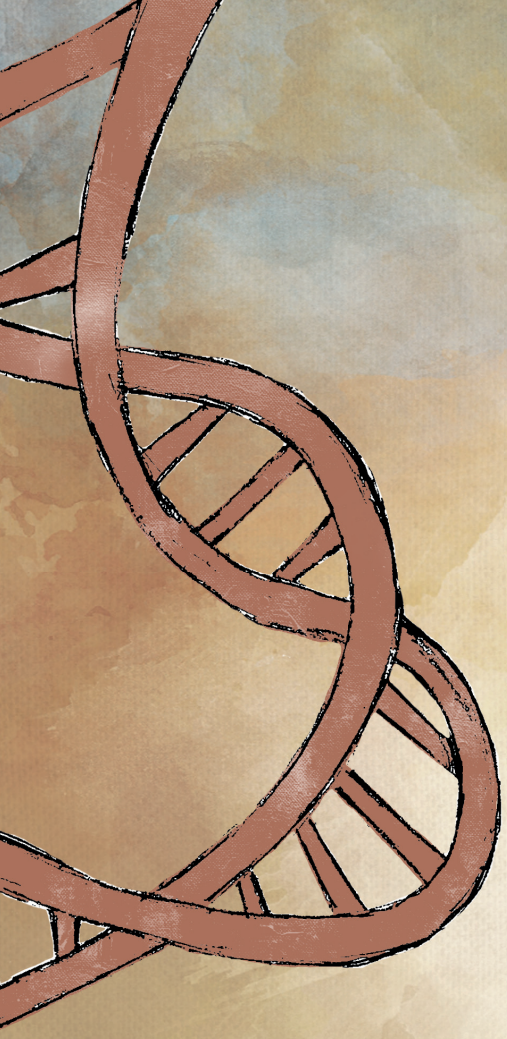

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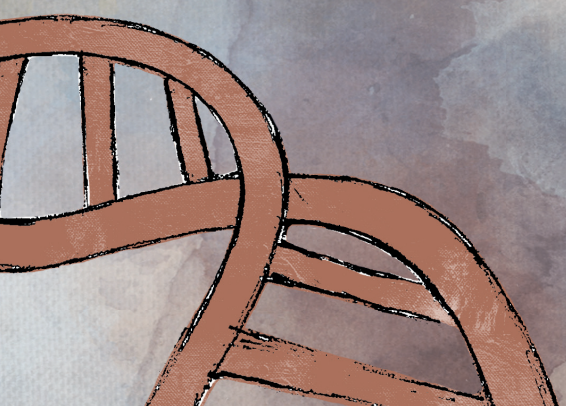
Introduction



Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterized by inflammation of synovial joints. The prevalence of RA in North America and Northern Europe is approximately 0.5-1.0%.[1] Data from Dutch general practitioners indicated a prevalence of 1.5% and over 12.000 new cases in the Netherlands in 2019.[2,3] Women are more often affected by the disease, approximately 75% of patients is female.[1] The etiology of RA is largely unknown, though several genetic and environmental risk factors have been established. The most important genetic risk factor is the human leukocyte antigen-shared epitope (HLA-SE),[4] the most well-known environmental risk factor is smoking.[1] Both factors associate predominantly with the development of autoantibody-positive RA.

Understanding the pathogenesis of RA is complicated by the heterogeneous origin of the disease. Largely, RA can be divided in autoantibody-positive and autoantibody-negative disease; in approximately 50% of early RA-patients autoantibodies are present.[1,5] The two autoantibodies that are generally acknowledged and most commonly used in clinical practice are rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA).

Though underlying biological mechanisms may be different, the clinical presentation of RA at diagnosis is similar between autoantibody-positive and autoantibody-negative disease.[6,7] Patients often present with symmetrical complaints of pain, stiffness and swelling in small joints of hands and feet, though large joints can also be involved. The presence of arthritis often leads to functional limitations, and prolonged inflammation of the joints may result in damage of the surrounding cartilage and bone. The disease may also cause systemic comorbidities, e.g. cardiovascular disease or infection, and increase mortality risk.[1] Joint damage and severe long-term outcomes predominantly associate with ACPA-positive RA.[7] In both ACPA-positive and ACPA-negative RA the disease may lead to work disability, extending consequences to the societal level. The economic burden is further increased by the high costs of medication, especially since the introduction of biologicals.[8]

Emerging therapies and extensive research have led to major improvements in the treatment of RA. Although in the past it was not exceptional for the disease to lead to extensive joint damage, nowadays this is often prevented. One major contributor to this improvement is the early recognition and treatment of the disease. It is recommended to start treatment with disease modifying anti-rheumatic drugs (DMARDs) within 12 weeks after presentation with joint swelling, during the so-called 'window of opportunity', hereby preventing full maturation of the disease

pathogenesis and irreversible damage and impairment.[9] Yet, it is unclear whether even earlier treatment could prevent the onset of clinical arthritis altogether. To fully elucidate when and how to interfere with the disease processes, it is necessary to further explore the phases comprising RA-development.

The stages of rheumatoid arthritis development

In the development of RA several phases can be discerned, as shown in Figure 1.[10,11] First of all, we can distinguish an asymptomatic (A-C) and a symptomatic phase (D-F). In the asymptomatic phase signs and symptoms of imminent joint disease are absent, though genetic and environmental risk factors may be present. Subsequent development of autoantibodies associated with RA, such as RF and ACPA, can occur in the asymptomatic phase.[12,13]

The symptomatic phase is characterized by the presence of joint symptoms and comprises of three clinical presentations. The first symptomatic phase (D) is characterized by complaints of the joints suggestive of rheumatic disease, but in absence of clinically apparent inflammatory arthritis (i.e. clinical joint swelling confirmed by physical examination of the rheumatologist). In the second symptomatic phase (E) clinical arthritis is apparent, though the disease cannot (yet) be classified as RA, and is therefore termed undifferentiated arthritis (UA). The third phase is RA (F), which can be classified according to the 1987 and/or 2010 American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria.[14,15] Importantly, not all patients progress through all phases during development of RA.

Figure 1. The phases of RA-development

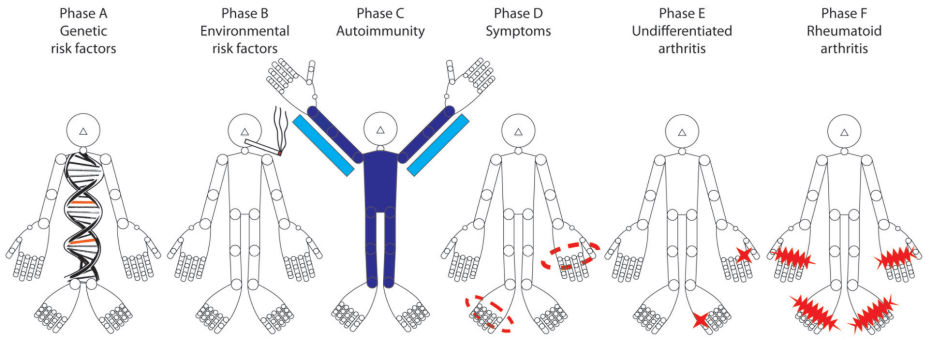


Image obtained from van Steenbergen et al.[10]

Over the years, a lot of research has been performed in UA and RA to establish the characteristics of patients developing the disease. This has improved the recognition and early treatment of these patients tremendously. Improvements have also been made in recognition of the disease even before presentation with arthritis. At-

risk populations have been defined by presence of certain risk factors, e.g. genetic predisposition or having a family member with RA, presence of autoantibodies, imaging findings or a distinct clinical presentation. Several trials are exploring, or have explored, treatment effects in at-risk populations.[16-22] Nevertheless, before preventive treatment can be implemented, risk stratification needs to be optimized. Presently a significant part of at-risk patients, despite presence of risk factors, will not develop RA. To avert preventive treatment in patients who would not develop RA after all (overtreatment), it is crucial to define an at-risk population with a high probability of developing RA. We therefore explore the beginning of the symptomatic phase; the moment when first contact between patient and physician is generally made.

Clinically suspect arthralgia

In line with Dutch guidelines for general practitioners, quick referral of patients with unexplained arthralgia, suspected arthritis or imminent rheumatic disease to the rheumatology outpatient clinic is encouraged.[23] Based on the clinical presentation and expertise of the rheumatologist, patients with arthralgia can further be classified as having clinically suspect arthralgia (CSA); this distinction has been shown to increase the chance of developing RA with an odds ratio (OR) of 55.[24] Patients with CSA often have arthralgia in small joints of the hands and feet for less than a year, which can be accompanied by morning stiffness and functional limitations. To standardize the definition of CSA, the EULAR definition of arthralgia suspicious for progression to RA was developed, see Table 1.[25] When applied in CSA-patients (as defined by the expertise and 'gut feeling' of the rheumatologist), presence of ≥3 out of 7 characteristics was shown to further double the risk for development of inflammatory arthritis.[26]

Table 1. EULAR characteristics describing arthralgia suspicious for progression to RA

History taking
Joint symptoms of recent onset (duration <1 year)
Symptoms located in MCP joints
Duration of morning stiffness ≥60 min.
Most severe symptoms present in the early morning
Presence of a first-degree relative with RA
Physical examination
Difficulty with making a fist
Positive squeeze test of MCP joints

Imaging

In addition to clinical aspects, imaging can be applied to further define patients at risk for progression to RA. Musculoskeletal ultrasound (US) and magnetic resonance imaging (MRI) can be used to detect subclinical inflammation, even before clinical arthritis becomes apparent.[27-29] Several inflammatory features can be distinguished; synovitis, tenosynovitis and bone marrow edema (BME), the latter only detectable with MRI. Presence of these inflammatory features is predictive for the development of clinically apparent inflammatory arthritis,[30,31] with tenosynovitis as the strongest predictor for progression.[31]

In addition to inflammatory features, imaging modalities can depict bone erosions. Bone erosions are a hallmark of RA, and are even in early arthritis frequently detectable on radiographs.[32] MRI is more sensitive than radiographs,[33] depicting small erosions even in symptom-free subjects.[34] Previous studies in early arthritis have identified several MRI-detected erosion characteristics specific for RA.[35] Even though the value of inflammatory features in CSA-patients has been extensively studied, there is no information on the predictive value of erosions for progression to RA.

While imaging modalities become more available and techniques continuously improve, the risk for overinterpretation of subclinical inflammation or erosions increases. MRI-detected erosions and inflammatory features are also present in symptom-free subjects from the general population.[34] This indicates that presence of a feature is not always indicative of (imminent) disease. Even presence of inflammatory features that are uncommon in symptom-free individuals cannot predict development of inflammatory arthritis with certainty; in some arthralgia patients these features may even spontaneously resolve.[36] Prescription of medication based solely on presence of subclinical inflammation may therefore lead to substantial overtreatment. This stresses the importance of further research improving the predictive value of imaging.

Furthermore, despite its advantages imaging is also costly, time consuming and requires training to consistently interpret the images. It is therefore valuable to investigate whether presence of subclinical inflammation can be estimated with clinical assessments.

Genetics and biomarkers

At-risk patients can also be distinguished by presence of genetic risk factors and serological and immunological markers. The most well-known genetic risk factor is found within genes encoding for HLA class II molecules; molecules involved in the presentation of antigens to T-cells. Several HLA-DRβ1 alleles that predispose for

RA have a similar amino acid sequence, the shared epitope, in the peptide binding groove of the HLA-DRβ1 molecule.[4] Presence of the HLA-SE is associated with ACPA-positive RA in particular.[37] In UA-patients the HLA-SE has been shown to independently associate with development of ACPA, and not with RA-development as such.[38] Predictive value of HLA-SE in autoantibody-positive arthralgia-patients were contrasting,[39,40] therefore the effect and value of HLA-SE in the phase of CSA is still unclear.

However, the value of ACPA and RF in the phase of CSA is extensively studied and clearer. Predominantly ACPA is highly predictive for development of RA,[31,39,41,42] RF was also associated with disease progression, though not independently from ACPA.[42] Nevertheless, of CSA-patients with both ACPA and RF, more than 30% does not develop clinical arthritis during two years of follow-up,[42] indicating that presence of these autoantibodies is also not fully indicative of (imminent) RA.

Over the years, other auto-antibodies associated with RA have been discovered. Similar to ACPA and RF, anti-carbamylated antibodies (anti-CarP) are also detectable years before disease onset and have been shown to associate with future RA,[43,44] though the added predictive value to ACPA and RF in arthralgia-patients remains questionable.[45,46] Anti-acetylated antibodies (AAPA) were highly specific (86%) for RA-patients, when compared to patients with persistent non-RA or resolving arthritis.[47] The presence and predictive value of AAPA have not yet been studied in the phase of CSA.

Still, the mere presence of autoantibodies does not yield 100% specificity; meaning autoantibodies can be present is subjects that do not develop RA. It is suggested that autoantibody-response maturation might be involved in progression from autoantibody-positivity to autoantibody-positive disease. Studies on RA-development have shown an increase in autoantibody levels, even before presentation of arthritis,[12,13,43] an increase in number of autoantibody isotypes,[48] expansion of the antigen recognition profile (epitope spreading),[49] and increased glycosylation within the variable domain of ACPA IgG during RA-development.[50] The exact timing of these events, whether they occur during the phase of CSA and their role in progression to clinical arthritis and RA remains to be elucidated.

Environmental factors

Closely linked to genetic factors and autoantibodies is environmental risk factor smoking. Similar to HLA-SE, smoking poses a high risk for autoantibody-positive RA in particular,[51] its effect influenced by the presence of HLA-SE.[52,53] Even though the risk of smoking has often been demonstrated in case-control studies, smoking was

not predictive for progression to RA in autoantibody-positive arthralgia and CSA-patients.[31,39,40] This might indicate that the risk imposed by smoking exerts its effect in another phase of RA-development.

Pathogenesis

Although many predictive factors have been discovered, thus far not a single factor, or combination of factors, can replace the rheumatologists judgement in establishing the diagnosis of RA. It is therefore important, in addition to the search for new (combinations of) predictive factors, to improve knowledge on disease pathogenesis and timing of risk factors during disease development. Knowing when certain factors are present, and when they exert their effect, may improve early diagnosis and optimize treatment targets in the different phases of RA-development.

Clinically suspect arthralgia cohort

The CSA-cohort, a longitudinal inception cohort started in 2012 at the rheumatology outpatient clinic of the Leiden University Medical Centre (LUMC), the Netherlands, was studied to answer the research questions of this thesis. Included patients had recent-onset (<1 year) arthralgia of small joints and were, based on the clinical expertise and pattern recognition of the rheumatologist, at risk for development of RA. Patients were excluded if clinical arthritis was already present, or if a different explanation for the joint pain was more likely, e.g. osteoarthritis or fibromyalgia. Since general practitioners in the area of Leiden are discouraged from performing autoantibody tests, autoantibody status was largely unknown at the time of inclusion; the CSA-cohort therefore comprises of both autoantibody-negative and autoantibody-positive patients.

Baseline visits consisted of physical examination, blood sampling, questionnaires and a contrast-enhanced 1.5T MRI of the hand (metacarpophalangeal (MCP) joints 2-5 and wrist) and foot (metatarsophalangeal (MTP) joints 1-5). MRIs were scored for presence of subclinical inflammation (synovitis, tenosynovitis and BME) and erosions in line with the RA MRI scoring system (RAMRIS) and Haavardsholm et al.,[54,55] and evaluated with symptom-free controls as reference.[34]

Patients were followed for two years, with scheduled visits at 4, 12 and 24 months. In case of an increase in symptoms, or suspected arthritis, additional visits were performed. During follow-up treatment with DMARDs (including corticosteroids) was not allowed. Follow-up ended after two years, or when the main outcome was reached; clinically apparent inflammatory arthritis (IA) as determined by physical examination of the rheumatologist.

Aims and outline of this thesis

This thesis has two main aims:

1. to improve prediction and early detection of rheumatoid arthritis
2. to improve understanding of pathogenesis underlying rheumatoid arthritis development

In **Part I** the predictive value of several clinical and imaging factors is evaluated in patients with CSA.

In **Chapter 2** the value of an easy clinical test, the ability of a patient to make a fist, is studied. Difficulties making a fist in CSA is considered a risk factor for the progression to IA, however, its predictive value has never been studied separately. In addition, the underlying cause of difficulties making a fist is evaluated by studying the presence of MRI-detected subclinical inflammation in patients presenting with and without fist problems.

Chapter 3 focusses on a second clinical test often used to quickly assess the presence of synovitis in hands and feet; the squeeze test. It was investigated whether the squeeze test in CSA, in absence of clinical arthritis, is able to detect presence of subclinical synovitis as measured with MRI. The predictive value of the squeeze test is evaluated as well.

Several factors have led to a growing number of patients already being treated before the onset of clinical arthritis. Among which the emergence of imaging in clinical practice, as well as research indicating the predictive value of subclinical inflammation in the development of RA. In **Chapter 4** the presence of subclinical synovitis as starting point for treatment with DMARDs is evaluated, as well as its potential for overtreatment.

The predictive value of subclinical inflammation is widely investigated. In addition to inflammation, MRI-detected erosions are also frequently observed in the phase of CSA. The predictive value of MRI-detected erosions is investigated in **Chapter 5**.

In **Part II** the underlying pathogenesis of RA is further explored.

Development of autoantibodies often occurs prior to diagnosis of RA. In **Chapter 6** autoantibody presence and autoantibody-response maturation in the symptomatic phase of CSA is investigated. The potential role of autoantibody-response maturation in progression to IA is evaluated by analyses of three autoantibodies (ACPA, anti-CarP

and AAPA) in three different isotypes (IgM, IgG and IgA) at two timepoints.

In **Chapter 7**, the genetic risk factor HLA-SE and the environmental risk factor smoking are investigated. In this chapter the timing of these factors and their relation with autoantibodies in the development of RA is evaluated by analyses of previously reported literature on asymptomatic individuals, and data from three cohorts with symptomatic at-risk individuals.

Finally, in **Chapter 8** the summary and general conclusions from this thesis are provided. In **Chapter 9** the summary and conclusions are provided in Dutch.

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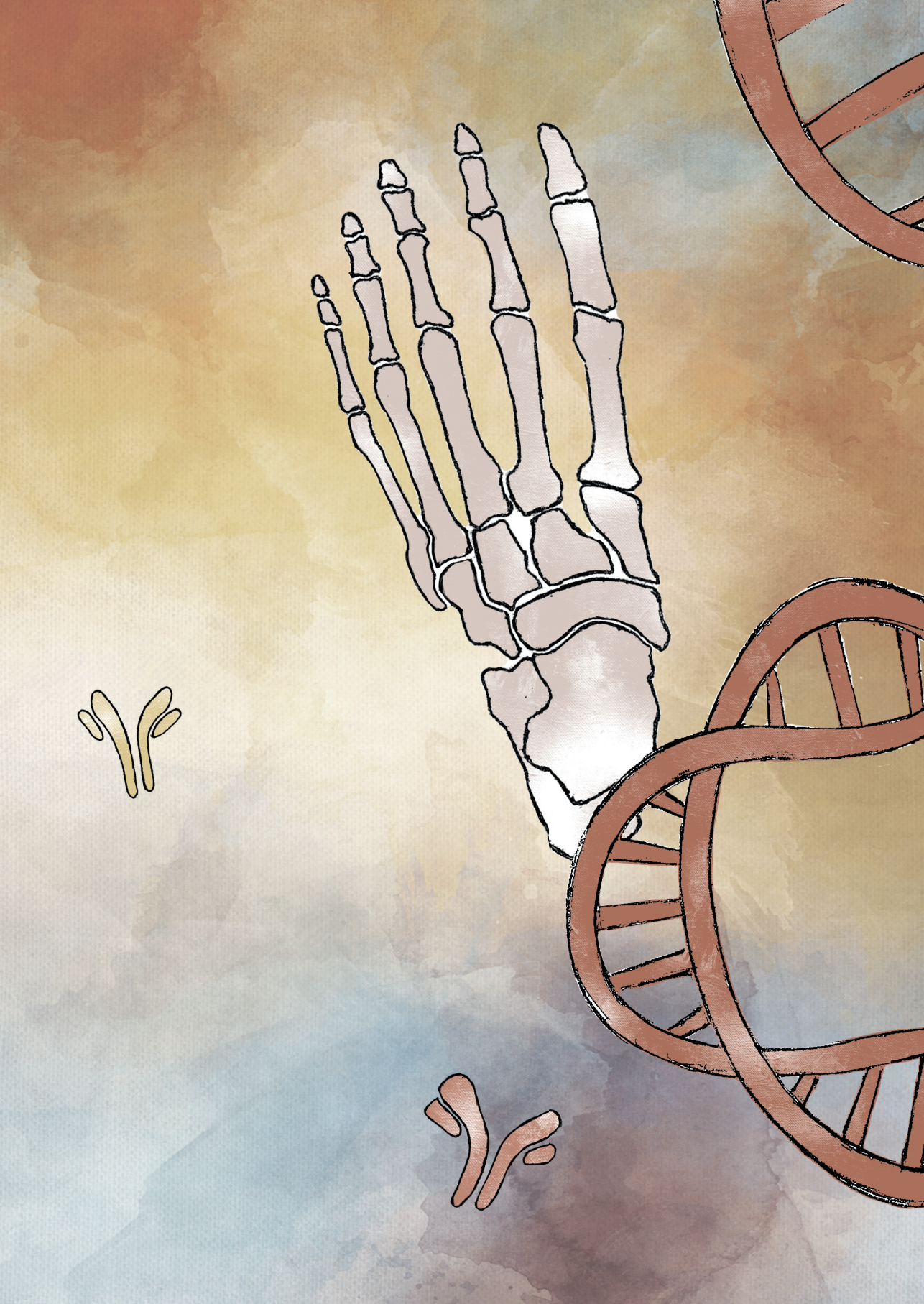

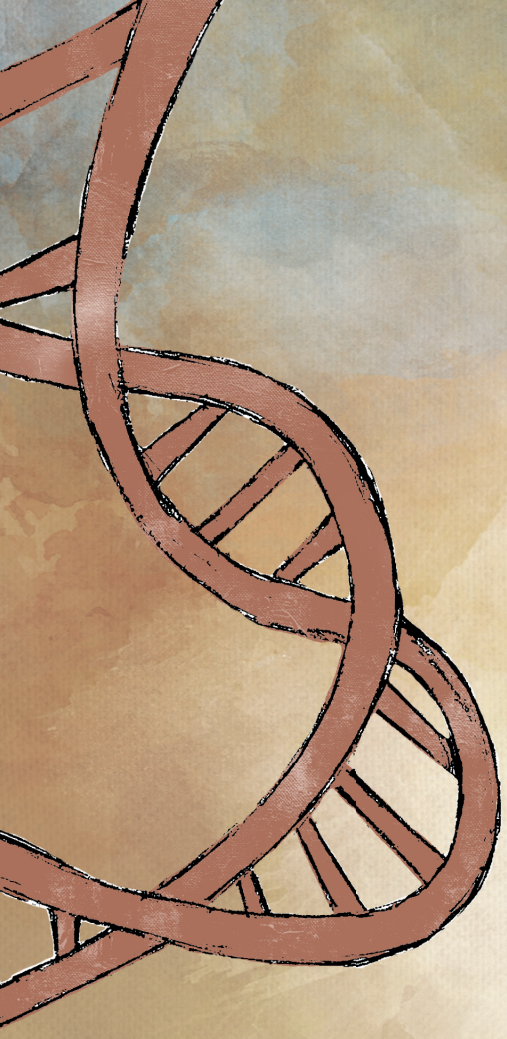

Prediction of Rheumatoid Arthritis

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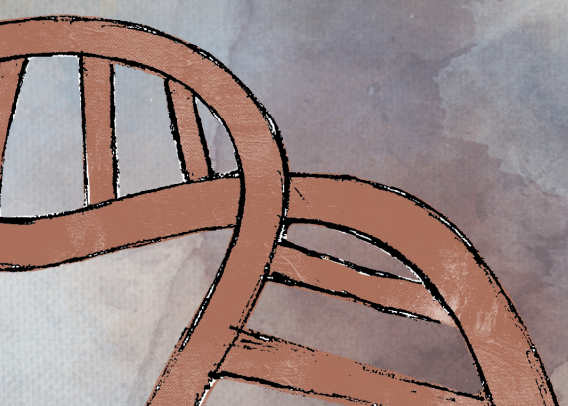

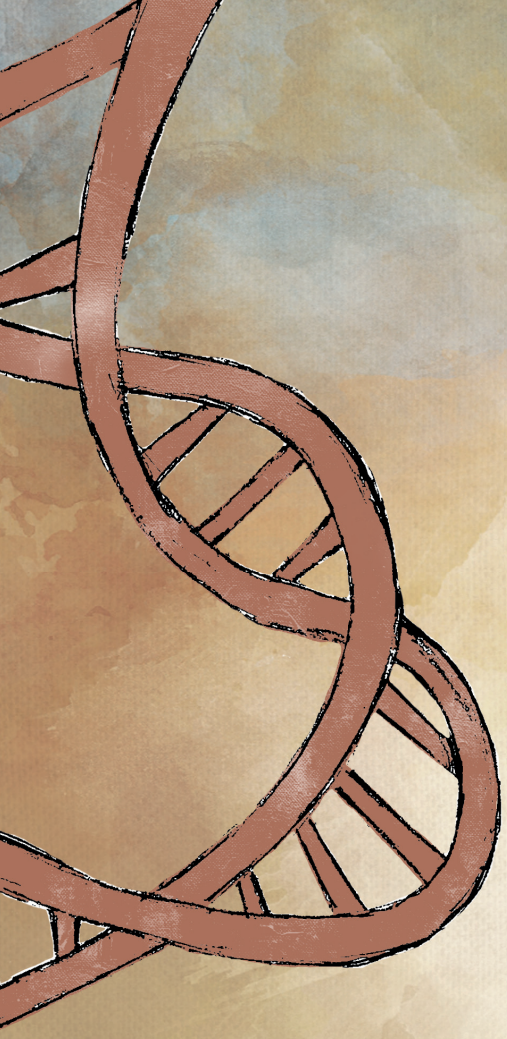

2

Difficulties making a fist in clinically suspect arthralgia; an easy applicable phenomenon predictive for RA that is related to flexor tenosynovitis

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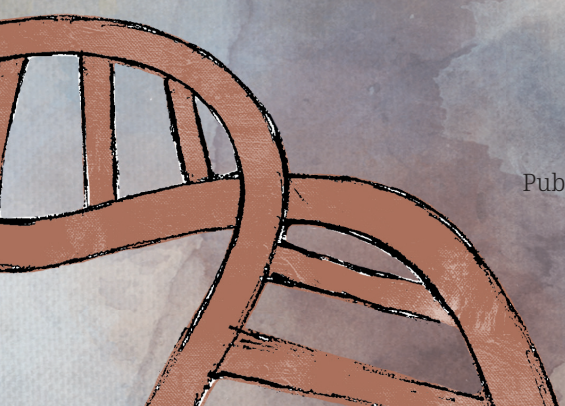

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Difficulties making a fist in patients presenting with recent-onset arthralgia of small joints without clinically detectable arthritis, is considered a risk factor for progression to inflammatory arthritis (IA) and Rheumatoid Arthritis (RA). This is also reflected by this sign being incorporated in the EULAR-definition of arthralgia suspicious for progression to RA.[1] However, to date there is barely scientific evidence for its predictive value and little comprehension on the underlying mechanism in recent-onset arthralgia. We studied if difficulties making a fist is indeed predictive for the development of IA and RA, and whether this sign is associated with subclinical inflammation.

Patients presenting with recent-onset (<1 year) arthralgia of the small joints were consecutively included in the Leiden Clinically Suspect Arthralgia (CSA)-cohort.[2] At baseline the ability to completely close the fist (actively close the fist with all fingertips touching the palm) and fist strength (measured by a patient squeezing the assessor's fingers) were determined (Figure 1). It was determined by trained research nurses in all patients, and for reliability purposes also by rheumatologists in a subset of patients. Contrast-enhanced 1.5T MRI of wrist and 2nd-5th metacarpophalangeal (MCP)-joints was performed and scored for synovitis, bone marrow edema (BME), tenosynovitis and MCP extensor peritendinitis. Patients were followed on development of clinically apparent IA, determined by rheumatologists (median follow-up 16 months (IQR 4-25)). Detailed description of the cohort, MRI-protocol and statistics are presented supplementary. Cox regression was performed with IA and RA (1987- or 2010-criteria-positivity) as primary and secondary outcome respectively; time-to-event was time from first presentation until IA-development. Associations between difficulties making a fist and subclinical inflammation in the same hand at baseline were assessed with logistic regression.

Flowchart and baseline characteristics are presented supplementary. From 606 CSA-patients, 86 (14%) had incomplete fist closure, 233 (38%) had decreased fist strength. In univariable Cox regression, the hazard ratio (HR) of incomplete fist closure was 2.22 (95% CI 1.36-3.64) and of decreased fist strength 1.33 (0.87-2.05). In multivariable analyses, corrected for age, gender, CRP- and ACPA-status, both signs were independently associated with IA-development; incomplete fist closure HR 2.33 (1.38-3.93) and decreased strength HR 1.62 (1.04-2.54). Similar findings were obtained with RA-development as outcome (Supplement).

To better understand the underlying pathology, as clinical arthritis was absent and therefore not the explanation, we evaluated whether fist problems were related to subclinical inflammation. Incomplete fist closure was associated with MCP flexor tenosynovitis and wrist flexor and extensor tenosynovitis in univariable analysis,

and MCP flexor tenosynovitis in multivariable analysis (OR 3.79 (2.04-7.04), Figure 1). Decreased fist strength was associated with MCP and wrist flexor tenosynovitis in univariable analysis, and wrist flexor tenosynovitis in multivariable analysis (OR 2.79 (1.52-5.12), Figure 1).

Finally the two tests were assessed by different observers in a subset of patients. Agreement was substantial for fist closure (n=324, Cohen's Kappa 0.61), but only fair for fist strength (n=318, Kappa 0.28; Supplement).

Difficulties making a fist in recent-onset arthralgia in the absence of clinically apparent arthritis is considered a sign of imminent RA. This is the first study providing scientific support for the predictive value of this sign; incomplete fist closure in particular had better reliability and higher predictive value.

Intuitively, assessment of fist strength (normal/decreased) by physicians who get pinched may be more subject to interobserver variation than visual evaluation if the fist is completely closed, this was illustrated by lower values of agreement. The lower reliability may also have contributed to lower HRs for fist strength.

The association between fist problems and tenosynovitis is plausible as tenosynovitis can hamper tendon gliding within its sheath, limiting tendon excursion and the ability to distribute muscle strength to the fingers. It is reasonable that fist closure was especially associated with MCP flexor tenosynovitis and fist strength with wrist flexor tenosynovitis as these respective tendons are important for these movements.

Thus, difficulties making a fist in CSA is a sign of underlying flexor tenosynovitis. Incomplete fist closure in particular is predictive for RA-development. In contrast to MRI, fist closure is simple to assess, also by physicians with little experience in joint examination. Therefore fist closure, a component of the EULAR definition of arthralgia suspicious for progression to RA,[1] is a feasible and valuable sign for use in daily clinical practice. However, as predictive values are dependent on prevalence, the value of this test in different patient populations (e.g. primary care) needs further investigation.

Figure 1. MRI-detected subclinical inflammation and associations with two components of difficulties making a fist, fist closure and fist strength, in patients with CSA.

	Fist closure		Fist strength	
				
	Complete	**Incomplete**	**Not decreased**	**Decreased**
	(n=483)	**(n=69)**	**(n=351)**	**(n=201)**
MCP	*Prevalence, n (%)*	*Prevalence, n (%)*	*Prevalence, n (%)*	*Prevalence, n (%)*
Flexor tenosynovitis	51 (10.6)	27 (39.1)	39 (11.1)	39 (19.4)
Extensor peritendinitis	30 (6.2)	4 (5.8)	23 (6.6)	11 (5.5)
Synovitis	62 (12.8)	12 (17.4)	40 (11.4)	34 (16.9)
BME	31 (6.4)	7 (10.1)	20 (5.7)	18 (9.0)
Wrist				
Flexor tenosynovitis	42 (8.7)	19 (27.5)	24 (6.8)	37 (18.4)
Extensor tenosynovitis	45 (9.3)	15 (21.7)	36 (10.3)	24 (11.9)
Synovitis	33 (6.8)	8 (11.6)	23 (6.6)	18 (9.0)
BME	65 (13.5)	9 (13.0)	46 (13.1)	28 (13.9)
	Univariable	**Multivariable [a]**	**Univariable**	**Multivariable [a]**
MCP	*OR (95% CI)*	*OR (95% CI)*	*OR (95% CI)*	*OR (95% CI)*
Flexor tenosynovitis	5.45 (3.10-9.57)**	3.79 (2.04-7.04)**	1.93 (1.19-3.12)**	1.33 (0.77-2.29)
Extensor peritendinitis	0.93 (0.32-2.72)	-	0.83 (0.39-1.73)	-
Synovitis	1.43 (0.73-2.81)	-	1.58 (0.97-2.60)*	1.37 (0.81-2.30)
BME	1.65 (0.70-3.90)	-	1.63 (0.84-3.16)	-
Wrist				
Flexor tenosynovitis	3.99 (2.16-7.39)**	1.76 (0.85-3.64)	3.07 (1.78-5.31)**	2.79 (1.52-5.12)**
Extensor tenosynovitis	2.70 (1.41-5.18)**	1.49 (0.71-3.14)	1.19 (0.69-2.05)	-
Synovitis	1.79 (0.79-4.05)	-	1.40 (0.74-2.67)	-
BME	0.97 (0.46-2.04)	-	1.07 (0.65-1.78)	-

At baseline data of difficulties making a fist and MRI-detected subclinical inflammation were available in 552 patients (see flowchart in the supplement).
[a] Multivariable logistic regression analyses were adjusted for age, gender and MRI inflammatory features from both wrist- and MCP joints with associations of p<0.1 in univariable logistic regression.
* p-value<0.1, ** p-value<0.01.
CSA: clinically suspect arthralgia, MCP: metacarpophalangeal joint, BME: bone marrow edema, OR: odds ratio, CI: confidence interval

Acknowledgements

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Supplementary File 1 – Detailed description of methods

Patients

Between April 2012 and October 2018, 613 patients were included in the Leiden CSA cohort. CSA-patients had recent-onset (<1 year) arthralgia in the small joints, which was likely to progress to RA based on the clinical expertise of the rheumatologist. Per definition, patients were excluded if arthritis was detected upon physical examination or if a different explanation for the joint pain (e.g. osteoarthritis, fibromyalgia) was more likely than imminent RA. Baseline visit consisted of physical examination, questionnaires, blood sampling and MRI. Follow-up visits were scheduled at 4, 12 and 24 months. When necessary, for instance in case of an increase of symptoms or when patients experienced joint swelling, additional visits were planned. Follow-up ended when patients developed arthritis (determined at physical examination of joints by the treating rheumatologist), or else after 2-years. The cohort has been described in detail previously.[1]

During follow-up treatment with disease-modifying antirheumatic drugs (DMARDs, including steroids) was not allowed. Since April 2015, CSA-patients with MRI-detected subclinical inflammation could participate in a randomized double-blind placebo-controlled trial (RCT; Treat Earlier, trial registration number: NTR4853), studying the effect of Methotrexate in preventing progression to RA. This RCT is still ongoing; patients enrolled in this trial (n=88) were excluded from longitudinal follow-up in the CSA cohort because of the 50% chance of DMARD-use (Supplementary Figure 1).

Assessment and reliability of difficulties making a fist

At baseline the ability to completely close the fist and fist strength were assessed in both hands. Complete fist closure was defined as the ability to actively close the fist, with all fingertips touching the palm, and was assessed by visual inspection. Fist strength was measured by trained research nurses (RNs) while a patient squeezed the 2nd and 3rd finger of the RN. To investigate reliability of the measures for fist closure and fist strength, tests were also performed and documented by rheumatologists in a subset of patients (n=324 and n=318, respectively). Measure of agreement between RN and rheumatologist were determined for both tests.

Fist strength can also be measured with a hand held dynamometer. Strength measured with a dynamometer in e.g. clinical trials is mainly used to evaluate continuous strength measures over time within persons, or to compare strength between groups. To use strength as a diagnostic factor within individuals, a single measure ought to be dichotomized according to norm values. Norm values have a wide range, also within

age and gender categories.[2] As single measures with a handheld dynamometer also introduce interobserver variation and reference values show a large distribution, reliability of the measure would remain questionable. Most importantly, as hand held dynamometers are not always available in clinical practice, the present used method reflects daily clinical practice best. For these reasons we chose not to use a handheld dynamometer.

MRI scanning and scoring protocol

Within two weeks after inclusion, CSA-patients underwent contrast-enhanced MRI of wrist and 2nd-5th metacarpophalangeal (MCP) joints of the most painful side (in case of equally severe symptoms on both sides, the dominant side was scanned).

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

The following sequence was acquired before contrast administration: T1-weighted fast spin-echo (FSE) sequence in the coronal plane (repetition time (TR) 575 ms, echo time (TE) 11.2 ms, acquisition matrix 388×288, echo train length (ETL) 2). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation (fatsat) in the coronal plane (TR/TE 700/9.7ms, acquisition matrix 364×224, ETL 2), T1-weighted FSE fatsat sequence in the axial plane (wrist: TR/TE 540/7.7 ms; acquisition matrix 320x192; ETL 2 and MCP-joints: TR/TE 570/7.7 ms; acquisition matrix 320x192; ETL 2).

Field-of-view was 100mm. Coronal sequences had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. Axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm with 20 slices for the wrist and 16 for the MCP-joints.

We used the contrast enhanced T1-weighted fat suppressed sequence to assess bone marrow edema (BME). According to the RA MRI scoring system (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, three previous studies have demonstrated that a contrast enhanced T1-weigthed fat suppressed sequence has a strong correlation with T2-weighted fat suppressed sequences.[3-5] Furthermore, the arthritis subcommittee of the European Society of Musculoskeletal Radiology (ESSR) also recommends the use of contrast enhanced T1-weighted fat suppressed sequences for depicting BME.[6] The T2-weighted image shows increased water signal and a contrast-enhanced T1-weighted sequence

shows increased water content and the increased perfusion and interstitial leakage. A strong correlation has been shown in arthritis patients and in patients without inflammatory diseases such as bone bruises, intraosseous ganglions, bone infarcts and even nonspecific cases.[4,5] Based on these results BME was assessed on contrast enhanced T1-weighted fat suppressed sequences as it has a higher signal to noise ratio and allowed a shorter scan time for patients.

All bones (with the exception of metacarpal base 1 and the trapezium), joints and tendons were scored semi-quantitatively according to the validated RAMRIS. Bones were scored separately for BME on a scale 0-3 based on the affected volume of the bone (no BME, >0-33%, >33-66%, >66%). Synovitis was scored on a range 0-3 based on the volume of enhancing tissue in the synovial compartment (none, mild, moderate, severe).[7] Similar to the scoring method described by Haavardsholm et al., tenosynovitis at the flexor and extensor sides of the wrist, flexor side of MCP joints and MCP extensor peritendinitis were scored based on the thickness of peritendinous effusion or synovial proliferation with contrast enhancement (normal, <2mm, 2-5mm, >5mm (range 0-3)).[8]

Scoring was performed independently by two trained readers. Interreader and intrareader intraclass correlation coefficients were ≥0.91 and ≥0.92, respectively.

MRI-detected subclinical inflammation

Mean scores from both readers were used to determine presence of subclinical inflammation (synovitis, BME, tenosynovitis and MCP extensor peritendinitis) for the wrist- and MCP-joints separately. As MRI-detected subclinical inflammation also can be present in the general population, scores were dichotomized with MRI-data of symptom-free controls as reference (n=193, as published previously).[9] Patients were considered positive for an inflammatory feature if it is uncommon in symptom-free controls, i.e. present in <5% of symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60).

Outcome

The main outcome for longitudinal analyses was development of clinical arthritis, determined by the rheumatologist at physical examination. The secondary outcome was RA-development (fulfilment of 1987- or 2010-criteria).[10,11]

Statistics

Cox regression was used to investigate predictive value of difficulties making a fist for the development of inflammatory arthritis (IA) and RA. Time-to-event was determined as the time from inclusion until the first time clinical IA (or RA) was observed by

the rheumatologist. Patients who did not develop IA (or RA) were censored at the date of their 2-year visit, or, when current follow-up was shorter than 2 years, at the date all medical files were last checked for IA (or RA) development (8 October 2018). Multivariable Cox regression was corrected for regular predictors (age, gender, CRP-status (normal/increased) and ACPA-status (anti-CCP2 positive/negative). Analyses were done with IA-development as primary outcome, and thereafter done with RA-development as outcome.

Associations between difficulties making a fist and subclinical inflammation in the same hand at baseline were assessed with logistic regression. Inflammatory MRI-features (from both wrist- and MCP joints) with associations of $p<0.1$ in univariable logistic regression were included together with age and gender in multivariable logistic regression.

The measure of agreement between RN and rheumatologists for tests of fist closure and fist strength was determined with the Cohen's Kappa.

P-values <0.05 were considered statistically significant. IBM SPSS Statistics Version 23 was used.

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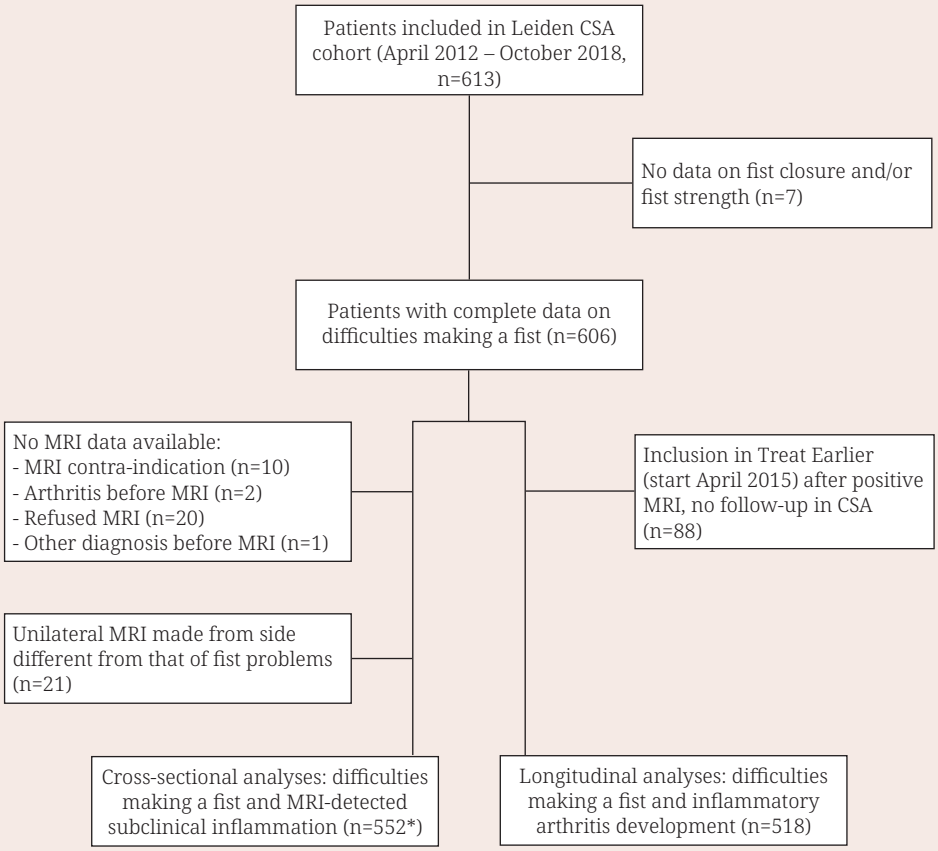
Supplementary Figure 1. Patient selection flowchart



Patients used for longitudinal analyses (n=518) had a median follow-up of 16 months (IQR 4-25), 85 (16%) developed inflammatory arthritis. *Fist closure and fist strength were observed by both RN and rheumatologist in 324 and 318 patients, respectively.

Supplementary Table 1. Baseline characteristics of the studied CSA-patients

	Cross-sectional: difficulties making a fist and MRI-detected subclinical inflammation (n=552)	**Longitudinal: difficulties making a fist and inflammatory arthritis development (n=518)**
Age in years, mean (SD)	44.0 (12.8)	43.5 (12.6)
Female, n (%)	411 (74.5)	404 (78.0)
Symptom duration in weeks, median (IQR)	19 (9-41)	19 (9-44)
68-TJC , median (IQR)	5 (2-10)	5 (2-10)
MRI inflammation score wrist and MCP, median (IQR)	1.8 (0.5-4.5)	1.5 (0.5-4.0)
ACPA positivity (≥7 U/mL), n (%)	76 (13.8)	68 (13.1)
RF positivity (≥3.5 IU/mL), n (%)	112 (20.3)	103 (19.9)
Increased CRP (≥5 mg/L), n (%)	117 (22.4)	104 (21.2)

CSA: clinically suspect arthralgia, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, ACPA: anti-citrullinated protein antibody, RF: rheumatoid factor, CRP: c-reactive protein

Supplementary Table 2. Predictive value of difficulties making a fist for the development of RA

	Univariable Cox regression		**Multivariable Cox regression**[a]	
	HR (95% CI)	*P-value*	*HR (95% CI)*	*P-value*
Incomplete fist closure with one or both hands	2.18 (1.22-3.90)	0.009	2.55 (1.38-4.71)	0.003
Decreased fist strength in one or both hands	1.29 (0.78-2.14)	0.328	1.76 (1.04-2.98)	0.036

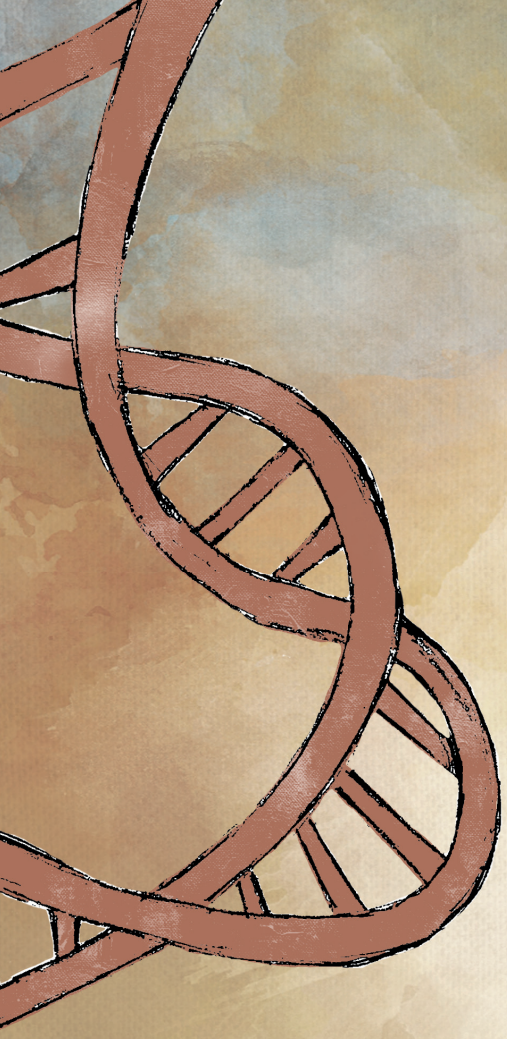
[a] Adjusted for age, gender, CRP-status and ACPA-status
RA: rheumatoid arthritis, HR: hazard ratio, CI: confidence interval

Supplementary Table 3. Measure of agreement between assessors of fist closure

Fist closure		**According to the rheumatologist**		
		Complete	Incomplete	Total
According to the research nurse	Complete	278	4	282
	Incomplete	20	22	42
	Total	298	26	324
Cohen's Kappa	0.61			

Supplementary Table 4. Measure of agreement between assessors of fist strength

Fist strength		**According to the rheumatologist**		
		Not decreased	Decreased	Total
According to the research nurse	Not decreased	163	23	186
	Decreased	81	51	132
	Total	244	74	318
Cohen's Kappa	0.28			



3

The value of the squeeze test for detection of subclinical synovitis in patients with arthralgia suspicious for progression to RA

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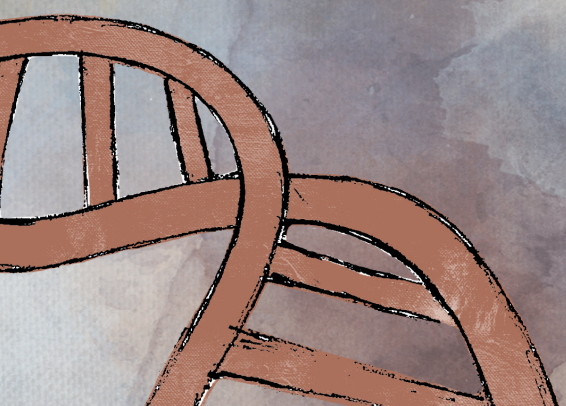
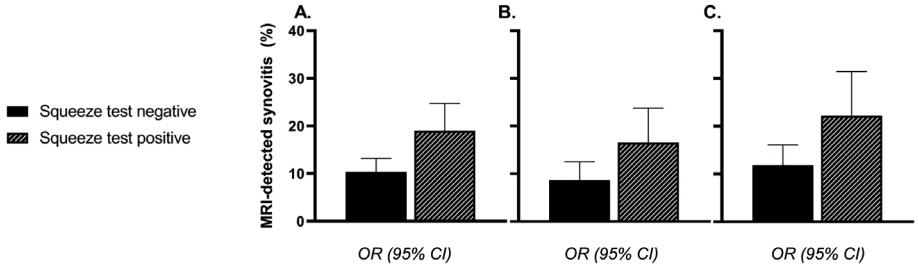


Sir, The squeeze test (or compression test) is often used to quickly screen for arthritis in metacarpophalangeal (MCP)- and metatarsophalangeal (MTP)-joints. A positive test is traditionally assumed to indicate presence of synovitis.[1] Previous studies in early arthritis indeed showed that a positive squeeze test was associated with presence of swollen MCP- and MTP-joints, as well as with local MRI-detected inflammation.[2] The sensitivity of the test, with MRI-detected synovitis as reference, was 31-33%.[2] The field of early arthritis is moving towards identifying patients at risk for rheumatoid arthritis (RA) in the phase of arthralgia. MRI-detected subclinical inflammation has been shown predictive for RA-development; of all inflammatory features, tenosynovitis had the strongest association.[3] We here aimed to assess if a positive squeeze test in patients with clinically suspect arthralgia (CSA) is associated with subclinical inflammation. We specifically hypothesized that it is associated with subclinical synovitis, in line with the original assumption of the test being a measure of synovitis. MRI-detected tenosynovitis was also studied, because we assumed that tenosynovitis at MCP- or MTP-level may also produce pain upon compression. Finally, we studied the association of the test with progression to inflammatory arthritis (IA).

Between April 2015-October 2018 315 patients were consecutively included in the Leiden CSA-cohort, details are provided supplementary. Inclusion criteria were recent-onset (<1 year) arthralgia of small joints and a clinical suspicion for progression to RA, which means that according to the pattern recognition of the rheumatologist at first visit, imminent RA was more likely than other diagnoses, as described previously.[4] At baseline the squeeze test was performed; compression across the knuckles of MCP- and MTP-joints with the force of a firm handshake, as described previously.[2] Unilateral contrast-enhanced 1.5T MRI of MCP(2-5)- and MTP(1-5)-joints was also made at baseline and scored by two trained readers for synovitis (according to RAMRIS[5]) and tenosynovitis (according to Haavardsholm[6]). MRI-scores were dichotomized with data from age-matched symptom-free controls as reference. A detailed scanning and scoring protocol and information on dichotomisation is provided supplementary. Follow-up ended when patients developed clinically apparent IA (determined at physical examination), or else after 2 years. Associations of the squeeze test and MRI-data (data of same extremity at baseline) were studied with generalized estimating equations, to account for the fact that in every patient a hand and a foot was assessed. The association of the squeeze test with IA-development was determined using cox regression.

Flowchart and baseline characteristics are shown supplementary (Supplementary Figure 1, Supplementary Table 1). 51% of CSA-patients had a positive squeeze test in MCP- or MTP-joints. In univariable analyses a positive test was associated with local subclinical synovitis (OR 2.10 (95%CI 1.30-3.40), Figure 1A) and tenosynovitis (OR 1.68

Figure 1. Association between the squeeze test and subclinical MRI-detected synovitis studied in A) MCP- and MTP-joints, B) MCP-joints only and C) MTP-joints only



	OR (95% CI)	OR (95% CI)	OR (95% CI)
Univariable	2.10 (1.30-3.40)	2.08 (1.02-4.22)	2.13 (1.10-4.12)
Multivariable [a]	1.90 (1.16-3.13)	2.04 (0.99-4.22)	1.83 (0.86-3.92)
Multivariable [b]	1.88 (1.14-3.11)	2.00 (0.96-4.16)	1.85 (0.86-3.99)

[a] Adjusted for tenosynovitis
[b] Adjusted for tenosynovitis, age and gender
Subclinical inflammation was considered present if the inflammatory feature was uncommon in symptom-free controls, i.e. present in <5% of symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60). Error bars represent 95%CI.
MCP: metacarpophalangeal, MTP: metatarsophalangeal, OR: odds ratio, CI: confidence interval

(1.05-2.68), Table S2). In multivariable analyses including both inflammatory features only synovitis remained significant (OR 1.90 (1.16-3.13), Figure 1A), also after further correction for age and gender (Figure 1A, Supplementary Table 2). Thus, a positive squeeze test is a measure of subclinical synovitis, with a sensitivity of 44% (95%CI 33-55) and specificity of 72% (68-76). When analysing MCP- and MTP-joints separately, the squeeze test was also associated with subclinical synovitis (OR 2.08 (1.02-4.22) and 2.13 (1.10-4.12) respectively in univariable analyses; Figure 1B-C, Supplementary Table 2).

A positive squeeze test in patients with CSA was not associated with IA-development in cox regression adjusted for age, gender, CRP and ACPA-status (HR 1.57 (0.77-3.19), Supplementary Table 3). This is consistent with the finding that subclinical synovitis was not associated with IA-development in multivariable analysis adjusted for age, gender, CRP, ACPA-status and subclinical tenosynovitis (HR 1.40 (0.59-3.31), whilst tenosynovitis was associated (HR 4.94 (2.03-12.06), Supplementary Table 4).

The squeeze test is known for its association with synovitis in patients with clinically manifest arthritis. This study is the first to investigate the association with subclinical inflammation in the phase of CSA. We demonstrated that a positive test was indeed associated with presence of subclinical synovitis. The sensitivity was 44%, this indicates that subclinical synovitis was frequently missed by the squeeze test. For certainty on presence of subclinical synovitis imaging could be used. However, whilst MRI is more sensitive for detection of subclinical synovitis, it is also invasive and

costly. In contrast, the squeeze test is easy to perform and free of costs, therefore it can provide value as a first screening tool.

The squeeze test of the MCPs is, in combination with other clinical characteristics, part of the EULAR-definition of arthralgia suspicious for progression to RA. This definition serves to identify this group of arthralgia patients and to distinguish them from arthralgia with other causes.[7,8]

A positive squeeze test within CSA was not significantly associated with IA-development. This may seem counterintuitive, as we have shown that it is a test for subclinical synovitis and subclinical inflammation is predictive for IA. However, this is explained by the fact that the latter association is mainly driven by tenosynovitis, as is shown previously.[3] Also in current data synovitis was not significantly associated with IA-development, in contrast to tenosynovitis.

In sum, the squeeze test is a simple test that, when positive in CSA, doubles the probability of presence of subclinical synovitis.

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Supplementary File 1 – Detailed description of methods

Patients

Between April 2015 and October 2018 315 patients were consecutively included in the Leiden Clinically Suspect Arthralgia (CSA)-cohort. Inclusion criteria were recent-onset (<1 year) arthralgia of small joints and clinical suspicion for progression to RA, which means that according to the pattern recognition of the rheumatologist at first visit, imminent RA was more likely than other diagnoses (e.g. osteoarthritis, fibromyalgia). Per definition, patients were excluded if arthritis was detected upon physical examination. At baseline visits physical examination was performed, questionnaires filled, blood samples taken and MRI performed. Physical examination included the squeeze test that was performed by rheumatologists; compression across the knuckles of metacarpophalangeal (MCP)- and metatarsophalangeal (MTP)-joints with the force of a firm handshake, it was considered positive if tenderness was induced, as described previously.[1] In line with national guidelines for general practitioners in the Netherlands, patients with suspected arthralgia or arthritis were referred to our outpatient clinic without antibody testing.[2] Therefore antibody status was mostly unknown during inclusion in the CSA-cohort, which took place at the first visit to the outpatient clinic. Thus at the time of the squeeze test rheumatologists were blind to this information. The MRI was made and scored blinded to any clinical data and rheumatologists were never informed on MRI-findings. Follow-up visits were scheduled at 4, 12 and 24 months. When necessary, for instance in case of an increase of symptoms or when patients experienced joint swelling, additional visits were planned. Follow-up ended when patients developed arthritis (determined at physical examination of joints by the treating rheumatologist), or else after 2 years. The cohort has been described in detail previously.[3]

During follow-up treatment with disease-modifying antirheumatic drugs (DMARDs, including steroids) was not allowed. However, CSA-patients with MRI-detected subclinical inflammation could participate in a randomized double-blind placebo-controlled trial (RCT; Treat Earlier, trial registration number: NTR4853), studying the effect of Methotrexate in preventing progression to RA. This RCT is still ongoing; patients enrolled in this trial (n=79) were excluded from longitudinal follow-up in the CSA cohort (Supplementary Figure 1).

MRI scanning and scoring protocol

Contrast-enhanced MRI was made of MCP(2-5)- and MTP(1-5)-joints of the most painful side (in case of equally severe symptoms on both sides, the dominant side was scanned). MRI was performed on a MSK-extreme 1.5T extremity MRI system (GE,

Wisconsin, USA) using a 145mm coil for the foot and a 100mm coil for the hand. The patient was positioned in a chair beside the scanner, with the hand or foot fixed in the coil with cushions.

In the hand (MCP-joints 2-5) the following sequence was acquired before contrast administration: T1-weighted fast spin-echo (FSE) sequence in the coronal plane (repetition time (TR) 575 ms, echo time (TE) 11.2 ms, acquisition matrix 388×288, echo train length (ETL) 2). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation (fatsat) in the coronal plane (TR/TE 700/9.7ms, acquisition matrix 364×224, ETL 2), T1-weighted FSE fatsat sequence in the axial plane (TR/TE 570/7.7 ms; acquisition matrix 320x192; ETL 2).

The obtained post-contrast sequences of the forefoot (MTP-joints 1-5) were: T1-weighted FSE fatsat sequence in the axial plane (TR/TE 700/9.5ms; acquisition matrix 364x224, ETL 2) and: T1-weighted FSE fatsat sequence in the coronal plane (perpendicular to the axis of the metatarsals) (TR/TE 540/7.5ms; acquisition matrix 320x192, ETL 2).

Field-of-view was 100mm for the hand and 140mm for the foot. Coronal sequences of the hand had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. Coronal sequences of the foot had 20 slices with a slice thickness of 3mm and a slice gap of 0.3mm. All axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm with 16 slices for the MCP-joints and 14 for the MTP-joints.

All joints were scored semi-quantitatively according to the validated RA MRI scoring system (RAMRIS). Synovitis was scored on a range 0-3 based on the volume of enhancing tissue in the synovial compartment (none, mild, moderate, severe).[4] Similar to methods described by Haavardsholm et al. the tenosynovitis-score was based on the thickness of peritendinous effusion or synovial proliferation with contrast enhancement (normal, <2mm, 2-5mm, >5mm (range 0-3)).[5]

Scoring was performed independently by two trained readers. Interreader and intrareader intraclass correlation coefficients were ≥0.91 and ≥0.92, respectively.

Mean scores from both readers were used, in case of disagreement between readers the lowest score was used. Then, as MRI-detected subclinical inflammation also can be present in the general population, scores were dichotomized with MRI-data of symptom-free controls as reference (n=193, as published previously).[6] Patients were

considered positive for an inflammatory feature (synovitis or tenosynovitis) if the feature (with the observed severity) was present in <5% of symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60).

Outcome

The main outcome for longitudinal analyses was development of clinically apparent inflammatory arthritis (IA), determined by the rheumatologist (who was blinded to MRI-data but not to other general laboratory investigations including auto-antibody status) at physical examination.

Statistics

First the association of a positive squeeze test (including data of hands and feet) with MRI-detected inflammation was studied with generalized estimating equations (GEE), accounting for the fact that every patient contributed both a hand and a foot to the analysis. In this analysis unilateral data (same side for MRI and squeeze test) was used. Multivariable GEE analyses were adjusted for age and gender. Subanalyses were performed for the squeeze test at only MCP- or only MTP-joints separately.

The predictive value of the squeeze test for development of IA was studied with cox regression. Time-to-event was determined as the time from inclusion until the first time IA was observed by the rheumatologist. Patients who did not develop IA were censored at the date of their 2-year visit, or, when current follow-up was shorter than 2 years, at the date of the last visit, or for patients that were still being followed at the date all medical files were last checked for IA development (28 December 2018). Multivariable cox regression was corrected for regular predictors age, gender, CRP and ACPA-status.

P-values <0.05 were considered statistically significant. IBM SPSS Statistics Version 25 was used.

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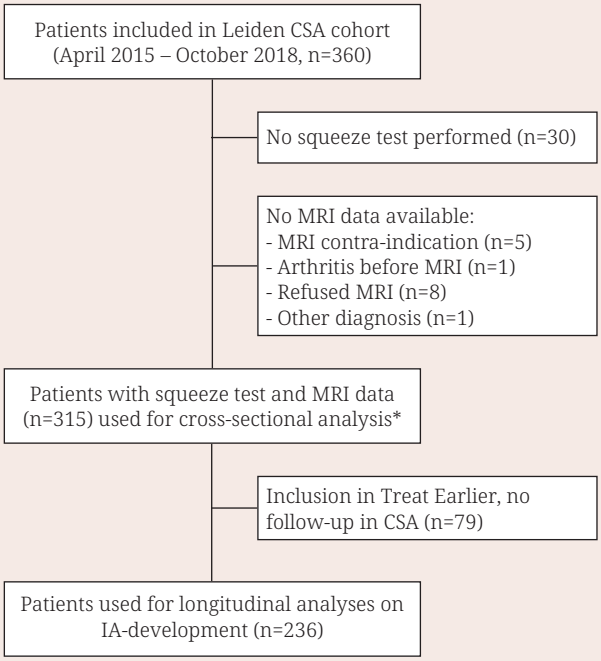
Supplementary Figure 1. Patient selection flowchart



Patients used for longitudinal analyses (n=236) had a median follow-up of 22 months (95% CI 20-24), 33 (14%) developed IA.
*11 hands or feet had no data of MRI and squeeze test at the same side and were not included in the analysis, hence the GEE included 619 extremities of 315 patients.

Supplementary Table 1. Baseline characteristics of the studied CSA-patients

	Total source population CSA-cohort (n=360)	Patients included in cross-sectional analysis: squeeze test and MRI inflammation (n=315)	Patients included in longitudinal analysis: squeeze test and IA development (n=236)
Age in years, mean (SD)	43.7 (12.4)	43.8 (12.3)	43.2 (12.0)
Female, n (%)	264 (73.3)	230 (73.0)	182 (77.1)
Symptom duration in weeks, median (IQR)	20 (8-48)	20 (9-46)	21 (10-50)
68-TJC , median (IQR)	5 (2-10)	5 (2-10)	5 (2-11)
ACPA positivity (≥7 U/mL), n (%)	52 (14.4)	46 (14.6)	31 (13.1)
RF positivity (≥3.5 IU/mL), n (%)	77 (21.4)	63 (20.0)	42 (17.8)
Increased CRP (≥5 mg/L), n (%)	77 (22.6)	66 (21.9)	41 (18.3)
Positive squeeze test MCP and/or MTP joints, n (%)	164 (49.7)	160 (50.8)	120 (50.8)
Presence of morning stiffness ≥60 minutes, n (%)	127 (36.2)	107 (35.0)	76 (32.9)
Family history positive for RA, n (%)	82 (23.2)	73 (23.3)	44 (18.8)

See the flowchart in Supplementary Figure 1 for the description of patient selection; patient characteristics were similar between the total source population and the studied groups, which argues against important selection bias.
CSA: clinically suspect arthralgia, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, ACPA: anti-citrullinated protein antibody, RF: rheumatoid factor, CRP: c-reactive protein, MCP: metacarpophalangeal, MTP: metatarsophalangeal, RA: rheumatoid arthritis

Supplementary Table 2. Associations between the squeeze test and MRI-detected subclinical inflammation in patients with CSA

Analyses on MCP- and MTP-joints	**Negative squeeze test (n=435)**	**Positive squeeze test (n=184)**	**Univariable**	**Multivariable[a]**	**Multivariable[b]**
	Prevalence, n (%)	*OR (95% CI)*	*OR (95% CI)*	*OR (95% CI)*	
Synovitis	45 (10.3)	35 (19.0)	2.10 (1.30-3.40)	1.90 (1.16-3.13)	1.88 (1.14-3.11)
Tenosynovitis	52 (12.0)	32 (17.4)	1.68 (1.05-2.68)	1.37 (0.85-2.22)	1.46 (0.89-2.38)
Analyses on MCP-joints only	**Negative squeeze test MCP (n=207)**	**Positive squeeze test MCP (n=103)**			
Synovitis	18 (8.7)	17 (16.5)	2.08 (1.02-4.22)	2.04 (0.98-4.25)	2.00 (0.96-4.16)
Tenosynovitis	34 (16.4)	20 (19.4)	1.23 (0.67-2.26)	1.06 (0.56-2.00)	1.12 (0.60-2.09)
Analyses on MTP-joints only	**Negative squeeze test MTP (n=228)**	**Positive squeeze test MTP (n=81)**			
Synovitis	27 (11.8)	18 (22.2)	2.13 (1.10-4.12)	1.83 (0.86-3.92)	1.85 (0.86-3.99)
Tenosynovitis	18 (7.9)	12 (14.8)	2.02 (0.93-4.40)	1.42 (0.58-3.50)	1.51 (0.61-3.73)

[a] Multivariable analyses including synovitis and tenosynovitis
[b] Multivariable analyses including synovitis, tenosynovitis, age and gender
CSA: clinically suspect arthralgia, MCP: metacarpophalangeal, MTP: metatarsophalangeal, OR: odds ratio, CI: confidence interval

Supplementary Table 3. Results from multivariable analysis including the squeeze test for the development of inflammatory arthritis

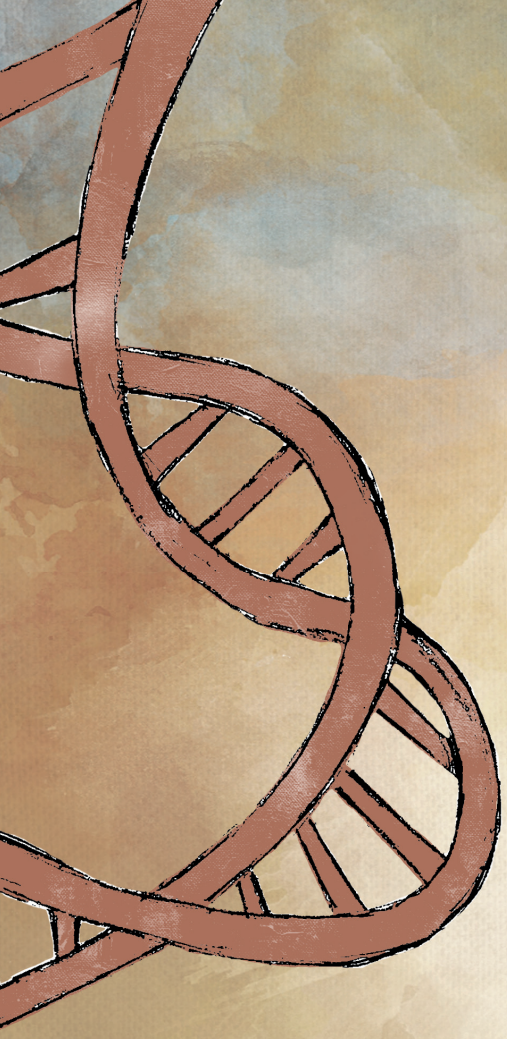
	Multivariable Cox regression	
	HR (95% CI)	*p-value*
Positive squeeze test	1.57 (0.77-3.19)	0.22
Age	1.02 (0.99-1.05)	0.27
Gender	1.61 (0.73-3.55)	0.24
Increased CRP	1.69 (0.77-3.74)	0.19
ACPA positivity	7.81 (3.77-16.2)	<0.001

73% of the patients that progressed to IA fulfilled the 2010 or 1987 criteria for RA at the time of IA-development, 3% were diagnosed with psoriatic arthritis and 24% had undifferentiated arthritis. HR: hazard ratio, CI: confidence interval, CRP: c-reactive protein, ACPA: anti-citrullinated protein antibody

Supplementary Table 4. Results from multivariable analysis including MRI-detected subclinical synovitis and tenosynovitis for the development of inflammatory arthritis

	Multivariable Cox regression	
	HR (95% CI)	*p-value*
MRI-detected synovitis	1.40 (0.59-3.31)	0.45
MRI-detected tenosynovitis	4.94 (2.03-12.06)	<0.001
Age	1.00 (0.97-1.03)	0.97
Gender	1.72 (0.79-3.75)	0.18
Increased CRP	1.41 (0.65-3.06)	0.38
ACPA positivity	4.17 (1.84-9.48)	0.001

HR: hazard ratio, CI: confidence interval, CRP: c-reactive protein, ACPA: anti-citrullinated protein antibody



4

Subclinical synovitis in arthralgia: how often does it result in clinical arthritis? Reflecting on starting points for disease-modifying anti-rheumatic drug treatment

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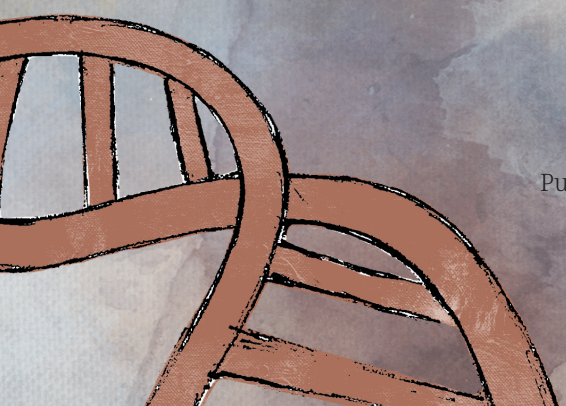

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Abstract

Objectives

According to guidelines, clinical arthritis is mandatory for diagnosing rheumatoid arthritis (RA). However, in the absence of clinical synovitis, imaging-detected subclinical synovitis is increasingly used instead, and considered as starting point for DMARD-therapy. To search for evidence, we studied the natural course of arthralgia-patients with subclinical synovitis from three longitudinal cohorts and determined the frequencies of non-progression to clinically apparent inflammatory arthritis (IA) (i.e. 'false-positives').

Methods

Subclinical synovitis in hands or feet of arthralgia-patients was visualized with ultrasound (two cohorts, subclinical synovitis definition: greyscale ≥2 and/or power doppler ≥1) or MRI (one cohort, definition: synovitis score ≥1 by two readers). Patients were followed for 1-year on IA-development; two cohorts also had 3-year data. Analyses were stratified for anti-citrullinated protein antibody (ACPA).

Results

Subclinical synovitis at presentation was present in 36%, 41% and 31% in the three cohorts. Of the ACPA-positive arthralgia-patients with subclinical synovitis 54%, 44% and 68%, respectively, did not develop IA. These percentages were even higher in the ACPA-negative arthralgia-patients: 66%, 85% and 89%. Similar results were seen after 3-years follow-up.

Conclusion

Replacing clinical arthritis by subclinical synovitis to identify RA introduces a high false positive rate (44-89%). These data suggest an overestimation regarding the value of ACPA-positivity in combination with the presence of subclinical synovitis in patients with arthralgia, which harbors the risk of overtreatment if DMARDs are initiated in the absence of clinical arthritis.

Introduction

Early start with disease modifying anti-rheumatic drugs (DMARDs) has become key in the treatment of rheumatoid arthritis (RA), because of its association with improved disease outcomes.[1] It has also fueled research that aims to identify patients at risk for RA in the symptomatic phase preceding clinically apparent arthritis, in the hope that even earlier treatment may prevent the development of RA. At present clinically apparent arthritis is mandatory for diagnosing RA and according to current guidelines it is the regular starting point for DMARD-treatment.[1]

However, this basic notion seems to be shifting at some places. A recent Dutch study showed that rheumatologist are increasingly willing to initiate 'preventive' treatment in the absence of clinical arthritis.[2] Likewise, a survey in the UK demonstrated that up to 73% of consulting rheumatologists would start DMARD-treatment in anti-citrullinated protein antibody (ACPA)-positive patients with musculoskeletal symptoms and power Doppler on ultrasound(US) in the absence of clinically apparent arthritis.[3]

Subclinical synovitis has indeed been consistently reported as a predictor for RA-development, however not all patients with this feature will develop RA.[4,5] Although others and we have published about predictive models, the risk of patients with subclinical synovitis to progress to RA, especially in the presence of ACPA , cannot be easily deduced from these studies, while this is the clinical situation were DMARDs are increasingly considered in clinical practice. Therefore, the question remains how often DMARD-treatment in such patients would be correct, and how frequently patients will be overtreated, because they would not have developed RA in the absence of DMARD-treatment.

It is also suggested to apply the 2010-classification criteria for RA in patients with subclinical inflammation, thus replacing the entry-criterion of clinical arthritis by subclinical synovitis. It is then conceptualized that subclinical synovitis and ≥6 points allow for an earlier classification of RA and could result in less overtreatment than treatment of subclinical synovitis alone.

Therefore, we set out to search for evidence of the natural course and determined in arthralgia-patients with subclinical synovitis, from three longitudinal cohorts, the frequencies of non-progression to clinically apparent inflammatory arthritis (IA) (i.e patients that could be considered as 'false-positives'), both in the presence and absence of ACPA. Furthermore, we explored if applying the 2010-criteria in patients with subclinical synovitis in the absence of clinical arthritis, thus broadening the entry-criterion, diminished the false-positive rate.

Methods

Cohorts

Data from three independent Dutch cohorts of arthralgia-patients with ≥1 year of follow-up for IA-development were used. The cohorts have been described previously.[6-8] Details of cohorts and imaging are presented in supplementary material.

In short, cohort 1 is the SONAR-study, a multicenter observational inflammatory arthralgia cohort. At baseline a bilateral ultrasound(US) was made of metacarpophalangeal(MCP)-joints 2-5, metatarsophalangeal(MTP)-joints 2-5 and wrists. Subclinical synovitis was defined as greyscale ≥2 and/or power doppler ≥1.

Cohort 2 is the clinically suspect arthralgia(CSA)-cohort. Patients underwent contrast-enhanced 1.5T MRI of the wrists, MCP 2-5 and MTP 2-5. Scans were independently scored by two trained readers for subclinical synovitis according to RAMRIS and a synovitis-score ≥1 by both readers was used as cutoff.[9]

Cohort 3 is the seropositive arthralgia cohort that included patients positive for ACPA and/or RF. A bilateral US of wrists, MCP 2-3 and MTP 2, 3 and 5 was made at baseline, according to a predefined US protocol.[4,7] Subclinical synovitis definition was similar to the SONAR study.

In all three cohorts the imaging examiners were blinded to the clinical details and the treating rheumatologists were blinded to the imaging results.

Outcome

The primary outcome of all three cohorts was development of IA after one year, determined by physical examination of the treating rheumatologist. In cohorts 2 and 3 the outcome was also assessed after three years. Importantly, DMARD treatment (including glucocorticoid injections) were not initiated in the phase of arthralgia and only prescribed after a patient had developed clinically apparent arthritis.

Analysis

The true and false positive rates were determined. These were respectively the percentages of patients that developed and did not develop IA, from all patients with a positive test. Analyses were stratified for ACPA-status. For our second aim we applied the 2010-criteria at baseline in patients with subclinical synovitis. The entry-criterion that requires presence of clinical arthritis was replaced by presence of ≥1 joint with subclinical synovitis in patients with arthralgia. The item 'number of involved joints' was solely based on the tender joint count (44-joints) and not by imaging.

Several sensitivity analyses were performed. First, the abovementioned analyses in cohort 2 and 3 were repeated when IA was assessed after 3-years of follow-up. Secondly, the definition of subclinical synovitis was evaluated in three ways. Because it is known that power Doppler could be a stronger predictor, progression to IA was shown for patients who had greyscale ≥2 or power doppler ≥1 separately.[6,10] In addition, multiple imaging studies in the general population showed that symptom-free persons can have inflammatory features.[11,12] Because this could affect the false-positive rate, analyses were repeated when features found in the general population were considered in the definition of the presence of subclinical synovitis. MRI detected subclinical synovitis was considered present if it occurred in <5% in the healthy population of the same age-category at the same joint (see Supplementary Methods for further explanation).[11,13] Similarly, the definition of US detected subclinical synovitis included the results from a large US study carried out on a symptom free population;[12] based on these results the cut-off value for MTP 2-3 was adjusted and subclinical synovitis was considered present in MTP 2-3 if GS≥3 and/or PD≥1, whilst the cut-off in MCP, wrist and MTP 4,5 joints remained unchanged (GS≥2 and/or power doppler ≥1). Finally, although the threshold for US detectable subclinical synovitis GS≥2 and/or PD≥1 is most frequently used in current literature,[4,6,10] we also evaluated the effect of a more stringent threshold (GS≥3 and/or PD≥2) on the false positivity rates.

STATA software V.15 and SPSS V25 were used.

Results

Baseline characteristics

166, 473 and 162 patients were included in cohort 1, 2 and 3. Table 1 presents the baseline characteristics. The percentage of ACPA-positives was 22% in cohort 1, 14% in cohort 2 and 56% in cohort 3. At baseline 36%, 41% and 31% of patients had subclinical synovitis. After one year 22%, 15% and 18% had developed IA, respectively.

False positive rates

Of the ACPA-positive patients with subclinical synovitis 54%, 44% and 68% did not develop IA in cohorts 1, 2 and 3 respectively (Figure 1A). In the ACPA-negative patients with subclinical synovitis, 66%, 85% and 89% did not progress to IA (Figure 1A).

Evaluation of use of subclinical synovitis as entry-criterion for the 2010-criteria

The analyses were also performed within arthralgia patients in whom subclinical synovitis was used as entry-criterion and who also had ≥6 points on the 2010-criteria

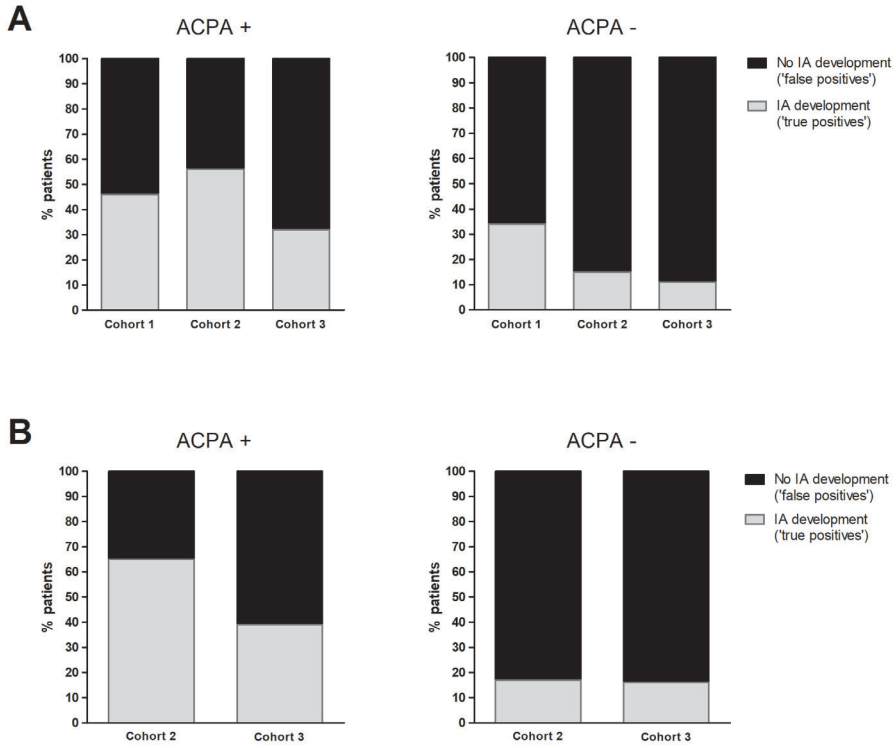
Table 1. Baseline characteristics of arthralgia-patients included in the three cohorts, also stratified for ACPA status

	All arthralgia patients			ACPA positive			ACPA negative		
	Cohort 1 (n=166)	*Cohort 2 (n=473)*	*Cohort 3 (n=162)*	*Cohort 1 (n=37)*	*Cohort 2 (n=64)*	*Cohort 3 (n=90)*	*Cohort 1 (n=129)*	*Cohort 2 (n=409)*	*Cohort 3 (n=72)*
Age in years, mean (SD)	45 (12)	44 (13)	51 (11)	45 (11)	48 (13)	51 (11)	45 (12)	43 (13)	52 (11)
Female, n (%)	136 (82)	366 (77)	120 (74)	32 (86)	52 (81)	67 (74)	104 (81)	314 (77)	53 (74)
Symptom duration in weeks, median (IQR)	29 (19-40)	19 (9-44)	57 (26-157)	28 (17-40)	24 (13-53)	52 (26-137)	29 (20-39)	18 (9-41)	83 (30-209)
TJC44, median (IQR)	5 (3-8)	5 (2-9)	1 (0-5)	4 (2-7)	3 (1-7)	1 (0-5)	5 (3-8)	5 (2-10)	1 (0-5)
ACPA positivity, n (%)	37 (22)	64 (14)	90 (56)	NA	NA	NA	NA	NA	NA
RF positivity, n (%)	49 (30)	95 (20)	119 (74)	22 (59)	49 (77)	47 (52)	27 (21)	46 (11)	72 (100)
Increased CRP, n (%)	39 (23)	101 (22)	12 (7)	11 (30)	20 (32)	8 (9)	28 (22)	81 (20)	4 (6)
Presence of local subclinical synovitis[a], n (%)	60 (36)	193 (41)	50 (31)	13 (35)	36 (56)	31 (34)	47 (36)	157 (38)	19 (26)

[a] Presence of Ultrasound (cohort 1 and cohort 3) or MRI (cohort 2) -detected subclinical synovitis. Joints screened for cohort 1 and cohort 2; metacarpophalangeal 2-5, radiocarpal, intercarpal, radioulnar (cohort 2) and metatarsophalangeal 2-5. Joints screened for cohort 3; metacarpophalangeal 2-3, metatarsophalangeal 2, 3, 5 and wrist.

Abbreviations: SD: standard deviation, IQR: interquartile range, TJC: tender joint count, ACPA: anti-citrullinated protein antibody, RF: rheumatoid factor, CRP: c-reactive protein, NA: not applicable

Figure 1. Percentage of arthralgia-patients with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year (A) and 3-years follow-up (B), stratified for ACPA-status.



(A) ACPA-positive patients; (cohort 1 n=37, cohort 2 n=64, cohort 3 n=90). Patients with subclinical synovitis at baseline; n=13, n=36, n=31, respectively. Of these n=6, n=20, n=10 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 1 n=129, cohort 2 n=409, cohort 3 n=72). Patients with subclinical synovitis at baseline; n=47, n=157, n=19, respectively. Of these n=16, n=23, n=2 patients developed IA after one year of follow-up, respectively.
(B) ACPA-positive patients; (cohort 2 n=43, cohort 3 n=90). Patients with subclinical synovitis at baseline; n=26, n=31, respectively. Of these n=17, n=12 patients developed IA after three years of follow-up, respectively. ACPA-negative patients; (cohort 2 n=292, cohort 3 n=72). Patients with subclinical synovitis at baseline; n=121, n=19, respectively. Of these n=20, n=3 patients developed IA after three years of follow-up, respectively.

(hereby imaging was not used to evaluate the number of involved joints). Within the ACPA-positive patients, 45%, 37% and 63% did not progress to IA (Supplementary Figure 1A). Within the ACPA-negative patients 67%, 82% and 89% did not progress. Hence in both ACPA-groups the false positive rates did not diminish when the 2010-criteria were used in arthralgia patients with subclinical synovitis.

Sensitivity analyses

First analyses were repeated for cohort 2 and 3 with IA-development after 3-year of follow-up. Similar false positive rates were observed (Figure 1B). Also the results of the use of the 2010-criteria in patients with subclinical synovitis after 3-years were similar (Supplementary Figure 1B).

Secondly, the results for progression to IA were shown separately for patients having greyscale ≥2 and patients having power doppler ≥1. No important differences were seen in patients having greyscale ≥2 compared to the main analysis (Supplementary Figure 2A/3A). The false positive rate for PD did diminish somewhat in subgroups of cohort 1 and 3 compared to the main analyses, but remained substantial (Supplementary Figure 2B/3B).

Additionally, imaging findings observed in symptom-free persons were considered in the definition of subclinical synovitis. When using a more stringent definition for MRI-detected synovitis 37% of ACPA-positive patients and 80% of ACPA-negative patients with subclinical synovitis did not progress to IA after 1 year (Supplementary Figure 4). Also when a more stringent definition for US-detected synovitis was used, a considerable proportion of ' false positives' remained, as 50% of the ACPA-positive and 71% of the ACPA-negative patients with subclinical synovitis did not progress to IA after 1 year (Supplementary Figure 5).

Finally, an even more stringent threshold for subclinical synovitis was studied (GS≥3 and/or PD≥2). Although the number of patients with arthralgia that had subclinical synovitis according to this definition decreased, the high false positive rates persisted (Supplementary Figure 6).

Discussion

Although daily practice most likely differs per region, there is an increasing tendency to start DMARD-treatment in arthralgia-patients with subclinical synovitis, at least in some places.[3] This is based upon the assumption that the clinical presentation of subclinical synovitis in ACPA-positive arthralgia is equivalent to imminent RA. The lack of evidence for this notion prompted us to perform a study in multiple cohorts. We observed that replacing clinical arthritis by subclinical synovitis for identification of IA introduced a high false-positive rate; as 44-68% of ACPA-positive and 66-89% of ACPA-negative arthralgia-patients with subclinical synovitis did not develop IA. These results on the natural disease course of arthralgia patients with subclinical synovitis imply that starting DMARD-treatment within these patients would lead to considerable overtreatment, as they would also not progress to IA without DMARD-therapy. Another argument is the lack of evidence that starting DMARD-treatment in this phase will prevent the development of RA. However, this is currently being investigated in several trials and is outside the scope of this study.[14]

Although the inclusion criteria of the three cohorts were somewhat different, the

primary results were comparable and this strengthens the validity of the results.

US and MRI are both suitable for detecting subclinical inflammation in arthralgia.[5,6,10] Although MRI is more sensitive than US, the decrease in sensitivity (with MRI as reference) is less for the detection of synovitis than for tenosynovitis and osteitis.[15,16] Interestingly, the results for the false positive rates of the two imaging modalities were not importantly different. However, for clinical purposes MRI can be less attractive since it is less easily available, is more expensive and requires intravenous contrast administration compared to US.[15] With respect to US, a limitation is that different machines were used in the two US studies. Nonetheless, the results were comparable and different machines are also used in daily clinical practice.

Ideally the definition of subclinical inflammation incorporates correction for the symptom-free population to prevent false-positive findings.[11,12] For MRI, reference values were available and considered. For US, we used the results of Padovano et al.[12] and the results with and without correction were similar. In, cohort 3 the false-positive rate reduced slightly but remained considerable. This suggests that signs of inflammation found in the normal population do not explain the observed false-positive rates.

The 2010-criteria are intended for classification and not for diagnosis/treatment start. Furthermore, to prevent false-positive classifications the 2010-criteria should only be applied in case of a clinical diagnosis of RA with ≥1 clinical swollen joint. Nonetheless in the 'pre-RA field' it is suggested that applying the 2010-criteria to patients with subclinical inflammation can be helpful. Previous studies that evaluated imaging as entry-criterion for the 2010-criteria were done in patients with clinically apparent arthritis or in mixed population with arthralgia and arthritis.[17,18] Our data from three cohorts with arthralgia patients and subclinical synovitis revealed that a high proportion of patients with subclinical synovitis and ≥6 points did not develop IA/RA. Consequently, there is currently no evidence to change the entry-criterion of clinical synovitis into subclinical synovitis, as the false positive rate remained substantial.

Furthermore the additional benefit of applying imaging in the 2010-criteria in patients with clinical arthritis to determine the number of involved joints has also been studied.[13] This is different from the current research where imaging detected subclinical synovitis replaces the entry-criterion of clinical arthritis.

In clinical practice, rheumatologists may be inclined to start DMARDs in ACPA-positive arthralgia-patients with subclinical synovitis. The current data from three cohorts suggests that ACPA-positivity in combination with subclinical synovitis is

overestimated in their ability to indicate the future development of IA/RA. Also in our sensitivity analysis where more stringent definitions of subclinical synovitis were used, high false positive rates remained. Altogether, this emphasizes the need for other biomarkers, in addition to ACPA and subclinical synovitis, to enhance risk stratification in patients with arthralgia. For example, imaging-detected tenosynovitis has been shown to be a better predictor than imaging-detected synovitis.[5,19] Combining imaging with other predictors (e.g. clinical, genetic and serological data) will presumably result in higher positive predictive values and true positive rates.[14,19]

A recent study on long-term outcomes of arthralgia-patients with subclinical inflammation that did not progress to IA showed that 33-38% of these patients, including those with ACPA-positivity, had symptom resolution.[20] Interestingly, this was also associated with reduction of subclinical inflammation, illustrating that a combination of symptoms, inflammation and presence of autoantibodies can be self-limiting.

In conclusion, our results showed that presence of subclinical synovitis and ACPA-positivity is not equal to RA-development. Therefore, in our view, further observational studies on the natural disease course are necessary to derive accurate and validated risk stratification for patients presenting with arthralgia. So that, when randomized clinical trials have shown that treatment of arthralgia patients prevents progression to IA, we can apply this treatment to the right patients and avoid significant overtreatment.

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Supplementary Methods

Details about inclusion, clinical examination and follow-up of the cohorts have been described in detail previously.[1-3]

Cohort 1 SONAR, Rotterdam The Netherlands

Design of cohort

The first cohort consisted of data from the sonographic evaluation of hands, feet and shoulders in patients with inflammatory arthralgia (SONAR) study.[1] This is a multicenter observational cohort to identify subclinical inflammation in patients with inflammatory arthralgia symptoms using ultrasound (US). Patients were followed for one year on the development of inflammatory arthritis (IA) with scheduled visits after 6 and 12 months. At each visit, patients were seen by the research nurse, who performed the physical examination and took blood samples. Observed soft tissue swelling was always confirmed as an arthritis by the treating rheumatologist. At baseline a bilateral US was made of metacarpophalangeal (MCP)-joints 2-5, metatarsophalangeal (MTP)-joints 2-5 and radiocarpal (RC) and intercarpal (IC) joints.

The medical ethics committee of Erasmus University Medical Center (Erasmus MC), Rotterdam, The Netherlands approved the study protocol (MEC-2010–353). Furthermore, the study was assessed for feasibility by the local ethical bodies of the other two participating hospitals (Maasstad Hospital and Vlietland Hospital). All patients gave written informed consent before inclusion.

ACPA-testing

ACPA levels (EliA cyclic citrullinated peptide (anti-CCP2), Phadia, Nieuwegein, the Netherlands) were tested in the hospital of inclusion. For Erasmus MC and Vlietland hospital ACPA levels were considered positive if levels ≥10U/mL, for Maasstad Hospital ACPA levels were considered positive if levels ≥5U/mL.

Imaging protocol (Ultrasound)

A MyLab60(Esaote, Genoa, Italy) with a high-frequency linear array probe(LA435, 10–18 MHz) was used. Two trained ultrasonographers, who were blinded for the clinical details, performed the US. To minimize inter-observer variability, the scanning was performed according to a standardized protocol with fixed patient position and scanning planes, in accordance to EULAR guidelines.[4]

Joints scanned for the detection of US abnormalities were metatarsophalangeal joints (MTP) 2–5 (dorsal aspect), metacarpophalangeal joints (MCP) 2–5 (dorsal and palmar aspects), and the wrist (radiocarpal and intercarpal joints). As advised a single midline

(longitudinal 12 o'clock position) scan perpendicular to the bone surface was used.[5]

Evaluation of the US images was done as recommended by a modified version of the previously developed OMERACT.[6] A semi-quantitative scoring system of Szkudlarek (0–3) was used for both greyscale (GS) and Power Doppler (PD) images. For GS, all joints were graded as follows: 0 = no capsular distention; 1 = hypoechoic material only at the level of the joint margins; 2 = partial distention of the whole capsule, which appears mostly concave or flat; and 3 = complete distention of the whole capsule, which appears mostly convex. PD was only measured if GS≥1 and was graded as follows: 0 = absent, 1 = mild single-vessel signal or isolated signal, 2 = moderate confluent vessels, and 3 = marked vessel signals in more than half of the intra-articular area.[7] Subclinical synovitis was defined as GS≥2 and/or PD≥1.

Cohort 2 CSA cohort, Leiden, The Netherlands

Design of cohort

The second cohort consisted of patients from the clinically suspect arthralgia (CSA)-cohort in Leiden.[3] Patients had recent-onset (<1 year) arthralgia of small joints and were, according to the clinical expertise and pattern recognition of the treating rheumatologist, suspected for progression to RA. Patients were followed for the development of IA with scheduled visits after 4, 12 and 24 months, with additional visits in between or thereafter if patients experienced an increase in symptoms. Patients underwent a contrast-enhanced 1.5T MRI of the wrist, MCP 2-5 and MTP 2-5 at baseline.

Ethics approval was obtained from the medical ethics committee of the Leiden University Medical Center, Leiden, The Netherlands.

ACPA-testing

ACPA levels (EliA cyclic citrullinated peptide (anti-CCP2), Phadia, Nieuwegein, the Netherlands) were considered positive if levels ≥7U/mL.

Imaging protocol (MRI)

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

In the hand (metacarpophalangeal (MCP) joints 2-5 and wrist) the following sequence was acquired before contrast administration: T1-weighted fast spin-echo (FSE) sequence in the coronal plane (repetition time (TR) 575 ms, echo time (TE) 11.2 ms, acquisition matrix 388×288, echo train length (ETL) 2). After intravenous injection

of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation (fatsat) in the coronal plane (TR/TE 700/9.7ms, acquisition matrix 364×224, ETL 2), T1-weighted FSE fatsat sequence in the axial plane (wrist: TR/TE 540/7.7 ms; acquisition matrix 320x192; ETL 2 and MCP-joints: TR/TE 570/7.7 ms; acquisition matrix 320x192; ETL 2).

The obtained sequences of the forefoot (metatarsophalangeal (MTP) joints 2-5) were for the first 77 patients before contrast administration: T1-weighted FSE sequence in the axial plane (TR/TE 650/17ms; acquisition matrix 388x288, ETL 2); and T2-weighted FSE fatsat sequence in the axial plane (TR/TE 3000/61.8; acquisition matrix 300x224, ETL 7). Imaging of the foot was initially limited to pre-contrast axial sequences. For the latter 396 patients post-contrast sequences were included: T1-weighted FSE fatsat sequence in the axial plane (TR/TE 700/9.5ms; acquisition matrix 364x224, ETL 2) and: T1-weighted FSE fatsat sequence in the coronal plane (perpendicular to the axis of the metatarsals) (TR/TE 540/7.5ms; acquisition matrix 320x192, ETL 2).

Field-of-view was 100mm for the hand and 140mm for the foot. Coronal sequences of the hand had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. Coronal sequences of the foot had 20 slices with a slice thickness of 3mm and a slice gap of 0.3mm. All axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm with 20 slices for the wrist, 16 for the metacarpophalangeal-joints and 14 for the foot.

All joints were scored semi-quantitatively according to the validated RA MRI scoring system (RAMRIS). Synovitis was scored on a range 0-3 based on the volume of enhancing tissue in the synovial compartment (none, mild, moderate, severe).[8]

Scoring was performed independently by two trained readers. Interreader and intrareader intraclass correlation coefficients were ≥0.91 and ≥0.92, respectively.

Mean scores from both readers were used. For the main analyses synovitis was considered present when at least one joint had a mean synovitis score of ≥1.

For the subanalysis concerning the symptom-free population, it is known that MRI-detected synovitis can also be present in the general population, scores were dichotomized with MRI-data of symptom-free controls as reference (n=193, as published previously).[9] Then, synovitis was considered present if the feature (with the observed severity) was present in <5% of symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60).

Cohort 3 Seropositive arthralgia cohort, Amsterdam, The Netherlands

Design of cohort

Data from the third cohort derived from Amsterdam was also described in detail previously.[2] This study consecutively included seropositive arthralgia-patients (positive for ACPA and/or RF) from March 2009 till December 2015. Patients were followed on IA-development with scheduled visits every 12 months and additional visits in case of suspected arthritis for up to 5 years. An US of bilateral wrists, MCP 2-3 and MTP 2, 3 and 5 was made at baseline.

Study was approved by the Slotervaart ethics committee. Signed informed consent was obtained from all patients prior to inclusion.

ACPA-testing

ACPA levels (EliA cyclic citrullinated peptide (anti-CCP2), Phadia, Nieuwegein, the Netherlands) were considered positive if levels ≥10U/mL.

Imaging protocol (Ultrasound)

The Acuson Antares ultrasound system, premium edition (Siemens, Malvern, PA, USA) using linear array transducers VF 13–5 SP for finger and toe joints (operating at 11.43 MHz for grayscale and 8.9 MHz for PD) and VF 13–5 for larger joints (operating at 11.43 MHz for grayscale and 7.3 MHz for PD), was used for all scans. A single radiologist experienced in musculoskeletal US, blinded to the clinical data, did all the US examinations. Joints scanned for the detection of US abnormalities were metatarsophalangeal joints (MTP) (dorsal site) 2, 3 & 5, metacarpophalangeal joints (MCP) 2–3 (palmar and dorsal site), and the wrists (radiocarpal and intercarpal joints and ulnocarpal joint including the ulnar styloid process). This was based on a predefined standard US protocol.[2,10]

The semi-quantitative scale (0–3) of Szkudlarek was used for both GS and PD images.[7] Subclinical synovitis was defined as GS≥2 and/or PD≥1.

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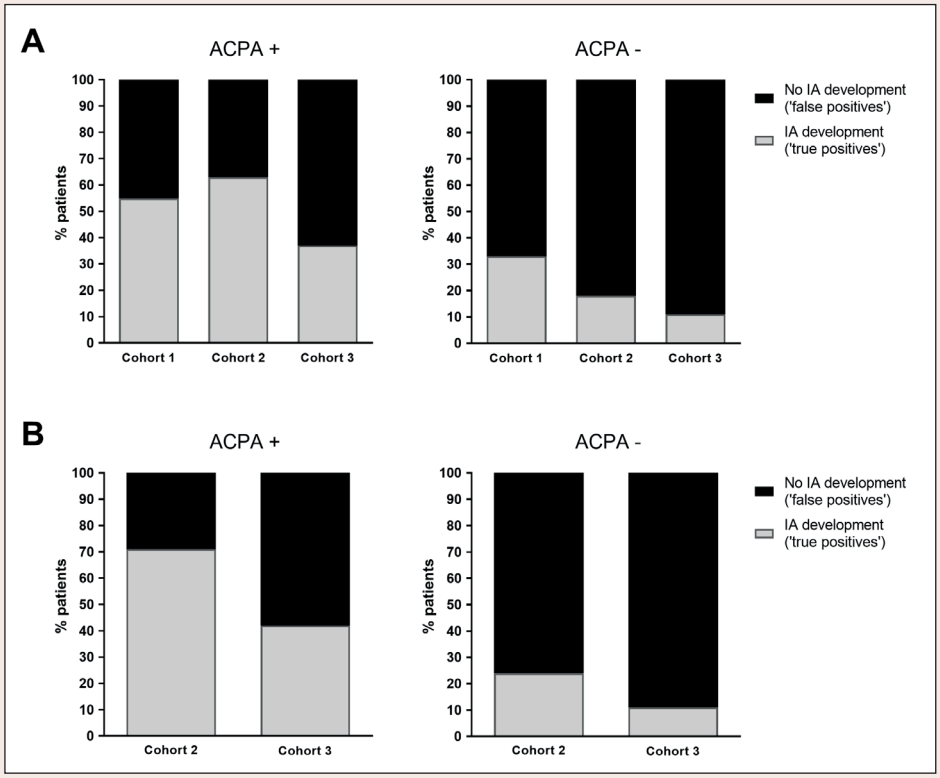
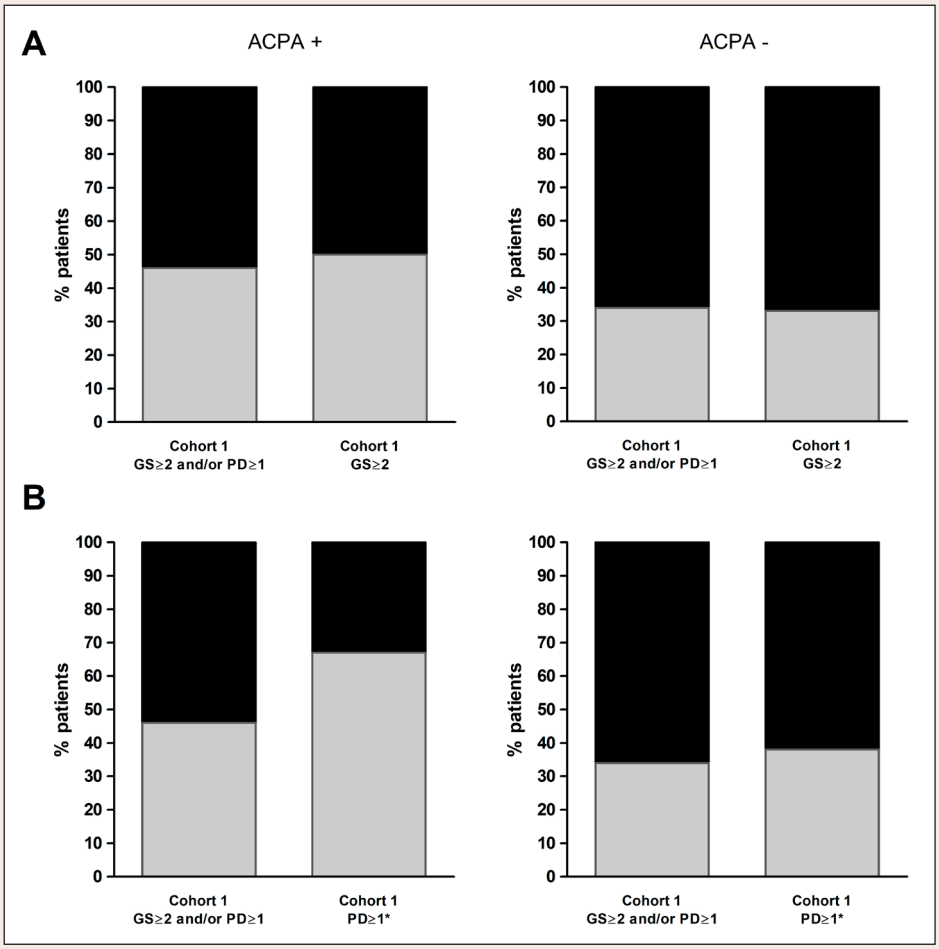
Supplementary Figure 1. Percentage of arthralgia-patients with subclinical synovitis (subclinical synovitis as entry-criterion) and ≥6 points on the 2010-criteria at baseline that did and did not develop inflammatory arthritis after 1-year (A) and 3-years follow-up (B) stratified for ACPA-status.



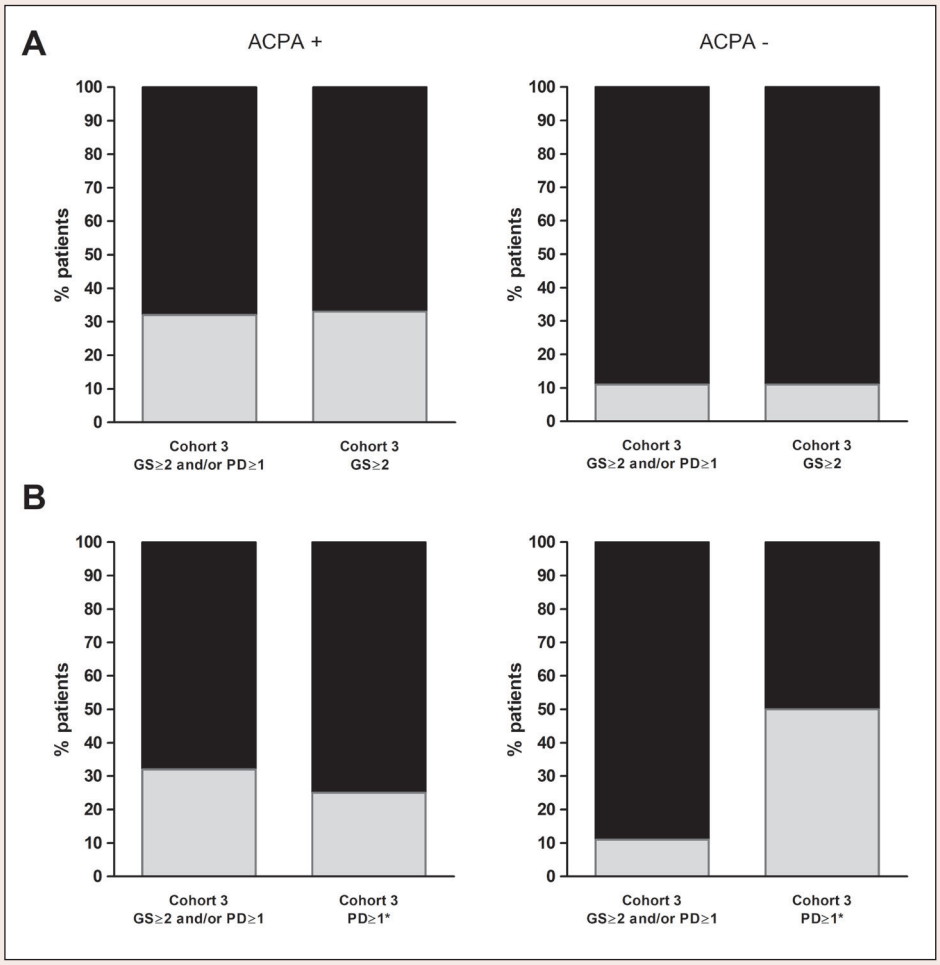
(A) ACPA-positive patients; (cohort 1 n=13, cohort 2 n=36, cohort 3 n=31). Patients with subclinical synovitis at baseline; n=11, n=27, n=19, respectively. Of these n=6, n=17, n=7 patients developed IA after one year of follow-up, respectively. ACPA-negative patients; (cohort 1 n=47, cohort 2 n=157, cohort 3 n=19). Patients with subclinical synovitis at baseline; n=12, n=39, n=9, respectively. Of these n=4, n=7, n=1 patients developed IA after one year of follow-up, respectively.
(B) ACPA-positive patients; (cohort 2 n=26, cohort 3 n=31). Patients with subclinical synovitis at baseline; n=21, n=19, respectively. Of these n=15, n=8 patients developed IA after three years of follow-up, respectively. ACPA-negative patients; (cohort 2 n=121, cohort 3 n=19). Patients with subclinical synovitis at baseline; n=29, n=9, respectively. Of these n=7, n=1 patients developed IA after three years of follow-up, respectively.

Supplementary Figure 2. Percentage of arthralgia-patients in cohort 1 with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year, stratified for ACPA, when subclinical synovitis was defined as grey scale ≥2 and/or power Doppler ≥1 (as in the main analyses) and when grey scale ≥2 or power Doppler ≥1 were used separately.



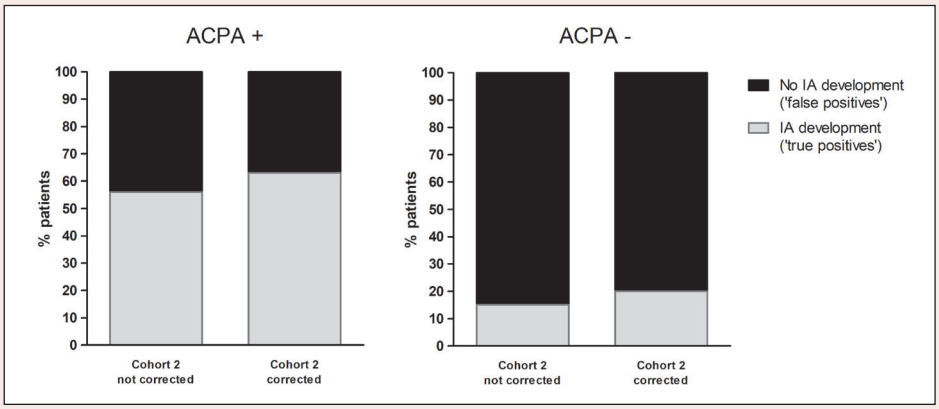
(A) ACPA-positive patients; (cohort 1 n= 37). Patients with subclinical synovitis at baseline; GS and/or PD n=13 , GS≥2 n=10. Of these n=6, n=5 patients developed IA after one year of follow-up, respectively. ACPA-negative patients; (cohort 1 n=129). Patients with subclinical synovitis at baseline; GS and/or PD n=47 , GS≥2 n=40. Of these n=16, n=13 patients developed IA after one year of follow-up, respectively.
(B) ACPA-positive patients; (cohort 1 n= 37). Patients with subclinical synovitis at baseline; GS and/or PD n=13 , PD≥1 n=9. Of these n=6, n=6 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 1 n=129). Patients with subclinical synovitis at baseline; GS and/or PD n=47 , PD≥1 n=16. Of these n=16, n=6 patients developed IA after one year follow-up, respectively.
* PD≥1 and GS=1

Supplementary Figure 3. Percentage of arthralgia-patients in cohort 3 with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year, stratified for ACPA, when subclinical synovitis was defined as grey scale ≥2 and/or power Doppler ≥1 (as in the main analyses) and when grey scale ≥2 or power Doppler ≥1 were used separately.



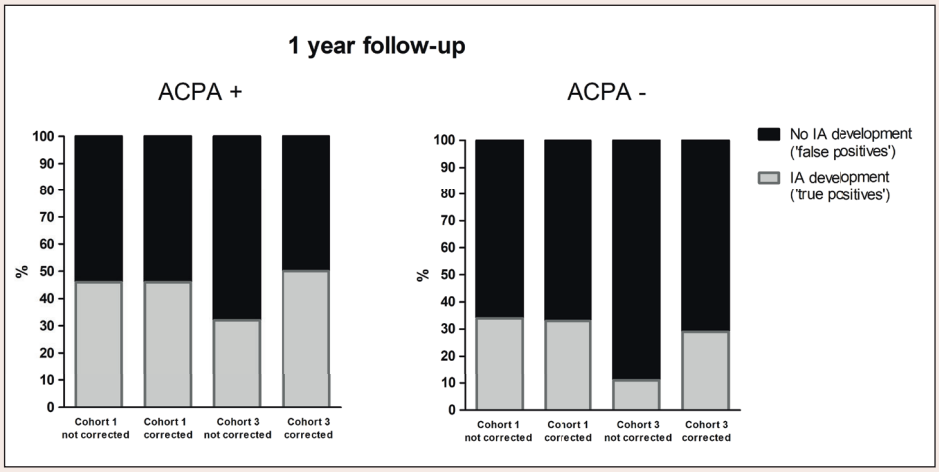
(A) ACPA-positive patients; (cohort 3 n=90). Patients with subclinical synovitis at baseline; GS and/or PD n=31 , GS≥2 n=30. Of these n=10, n=10 patients developed IA after one year of follow-up, respectively. ACPA-negative patients; (cohort 3 n=72). Patients with subclinical synovitis at baseline; GS and/or PD n=19 , GS≥2 n=19. Of these n=2, n=2 patients developed IA after one year of follow-up, respectively.
(B) ACPA-positive patients; (cohort 3 n=90). Patients with subclinical synovitis at baseline; GS and/or PD n=31 , PD≥1 alone n=4. Of these n=10, n=1 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 3 n=72). Patients with subclinical synovitis at baseline; GS and/or PD n=19 , PD≥1 n=2. Of these n=2, n=1 patients developed IA after one year follow-up, respectively.
* PD≥1 and GS=1

Supplementary Figure 4. Percentage of arthralgia-patients with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year, stratified for ACPA, before and after correcting the definition of subclinical synovitis for MRI-findings obtained in an age-matched symptom-free population.



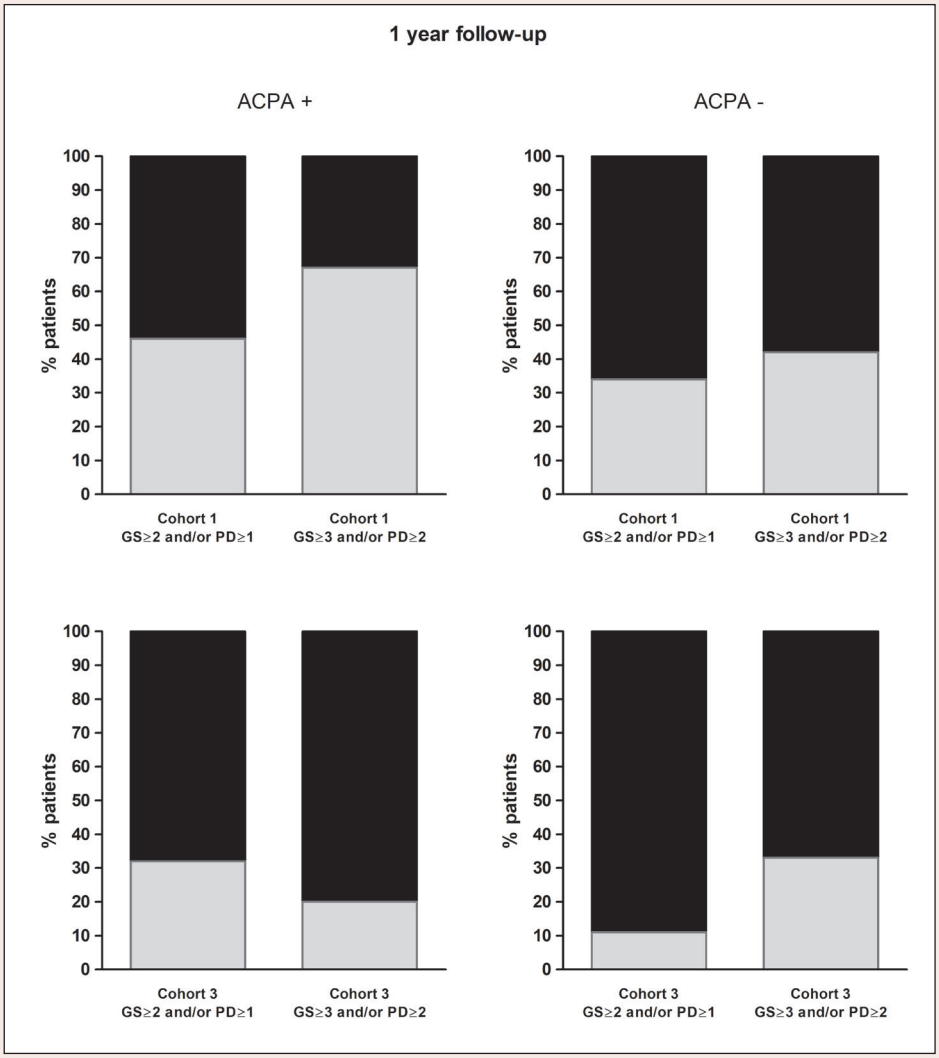
ACPA-positive patients; (cohort 2 n=64). Patients with subclinical synovitis at baseline; not corrected n=36, corrected n=24. Of these n=20, n=15 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 2 n=409). Patients with subclinical synovitis at baseline; not corrected n=157, corrected n=70, respectively. Of these n=23, n=14 patients developed IA after one year follow-up, respectively.

Supplementary Figure 5. Percentage of arthralgia-patients with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year, stratified for ACPA, before and after correcting the definition of subclinical synovitis for US-findings obtained in a symptom-free population.

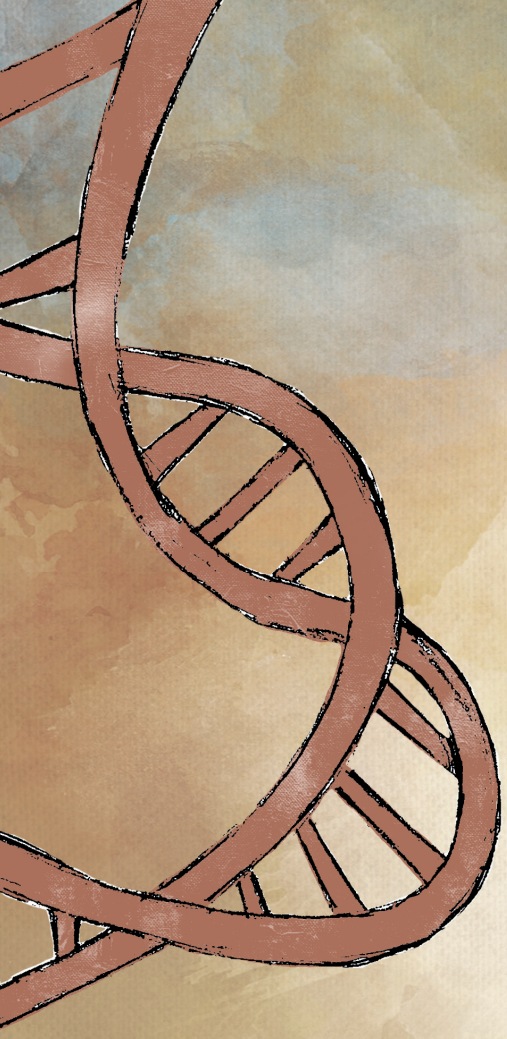


ACPA-positive patients; (cohort 1 n=37). Patients with subclinical synovitis at baseline; n=13 not corrected, n=13 corrected. Of these n=6, n=6 patients developed IA after one year follow-up, respectively. (Cohort 3 n=90). Patients with subclinical synovitis at baseline; n=31 not corrected, n=16 corrected. Of these n=10, n=8 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 1 n=129). Patients with subclinical synovitis at baseline; n=47 not corrected, n=42 corrected. Of these n=16, n=14 patients developed IA after one year follow-up, respectively. (Cohort 3 n=72). Patients with subclinical synovitis at baseline; n=19 not corrected, n=7 corrected, respectively. Of these n=2, n=2 patients developed IA after one year follow-up, respectively.

Supplementary Figure 6. Percentage of arthralgia-patients in two independent cohorts with subclinical synovitis at baseline that did and did not develop inflammatory arthritis after 1-year, stratified for ACPA, when subclinical synovitis was defined as grey scale ≥2 and/or power Doppler ≥1 (as in the main analyses) and as grey scale ≥3 and/or power Doppler ≥2 (more stringent threshold).



(A) ACPA-positive patients; (cohort 1 n=37). Patients with subclinical synovitis at baseline; GS≥2/PD≥1 n=13, GS≥3/PD≥2 n=6. Of these n=6, n=4 patients developed IA after one year of follow-up, respectively. ACPA-negative patients; (cohort 1 n=129). Patients with subclinical synovitis at baseline; GS≥2/PD≥1 n=47, GS≥3/PD≥2 n=12. Of these n=16, n=5 patients developed IA after one year follow-up, respectively.
(B) ACPA-positive patients; (cohort 3 n=90). Patients with subclinical synovitis at baseline; GS≥2/PD≥1 n=31, GS≥3/PD≥2 n=5. Of these n=10, n=1 patients developed IA after one year follow-up, respectively. ACPA-negative patients; (cohort 3 n=72). Patients with subclinical synovitis at baseline; GS≥2/PD≥1 n=19, GS≥3/PD≥2 n=3. Of these n=2, n=1 patients developed IA after one year follow-up, respectively.



5

Do magnetic resonance imaging-detected erosions predict progression to rheumatoid arthritis in patients presenting with clinically suspect arthralgia? A longitudinal study

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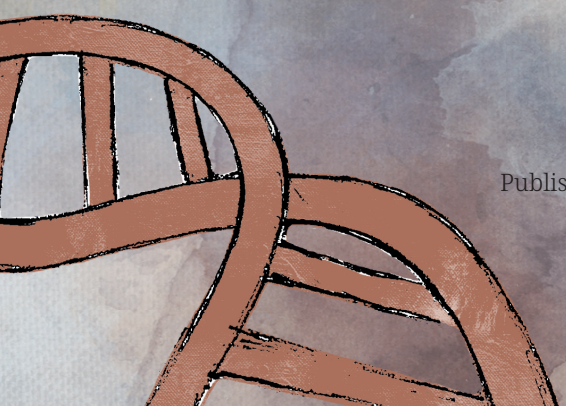

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Abstract

Objectives

Radiographic joint erosions are a hallmark of rheumatoid arthritis (RA). MRI is more sensitive than radiographs in detecting erosions. It is unknown if MRI-detected erosions are predictive for RA-development in patients with clinically suspect arthralgia (CSA). Therefore we investigated the prognostic value of MRI-detected erosions, defined as any MRI-erosion, or MRI-erosion characteristics that were recently identified as specific for RA in patients with evident arthritis.

Methods

Patients presenting with CSA (n=490) underwent contrast-enhanced 1.5T MRI of the wrist, metacarpophalangeal (MCP) and metatarsophalangeal (MTP) joints. MRIs were scored according to RAMRIS. Presence of any MRI-erosion (present in <5% of symptom-free controls) and RA-specific erosion characteristics as identified previously (grade ≥2 erosions, erosions in MTP5, erosions in MTP1 if aged <40) were studied with clinically apparent inflammatory arthritis development as outcome. Analyses were corrected for age and MRI-detected subclinical inflammation.

Results

Erosions were present in 20%. Presence of any MRI-erosion was not associated with arthritis development (HR multivariable analysis 0.97 (95% CI 0.59-1.59)). Also the different RA-specific erosion characteristics were not predictive (grade ≥2 HR 1.05 (0.33-3.34), erosions in MTP5 HR 1.08 (0.47-2.48) and MTP1 if aged <40 HR 1.11 (0.26-4.70)). Erosion scores were higher in ACPA-positive than in ACPA-negative patients (median 2.0 versus 1.0, p=0.002), and related to more subclinical inflammation. Within both subgroups, MRI-erosions were not predictive.

Conclusions

MRI-detected erosions in hands and feet were not predictive for inflammatory arthritis development. Therefore, evaluating MRI for erosions in addition to subclinical inflammation does not provide added clinical value in CSA.

Introduction

Rheumatoid arthritis (RA) is characterized by inflammation of synovial joints and subsequent bone damage. Bone erosions are frequently detectable at radiographs, even in an early disease phase.[1] Currently a lot of effort is undertaken to diagnose RA very early and imaging is increasingly used in prompt identification of RA. Moreover, a focus in research shifts towards identification of patients that will progress to RA already in the phase of arthralgia. Magnetic resonance imaging (MRI) is sensitive in detecting subclinical joint inflammation,[2] which is an established predictor for RA-development.[3] The value of different types of inflammatory features (synovitis, tenosynovitis and bone marrow edema (BME)) has been investigated; from these inflammatory features tenosynovitis has been shown to be most predictive for disease progression.[3] MRI also provides information on bone erosions. Thus far it is unknown if MRI-detected erosions are also predictive for progression to clinically apparent inflammatory arthritis (IA) and RA. However, we hypothesize that erosions might reflect previous episodes of early subclinical inflammation and hereby possibly provide additional value for prediction of IA- and RA-development.

The sensitivity of MRI to depict erosions is higher than that of radiographs.[4] Recent studies revealed that small MRI-detected erosions in hand and foot joints are also present in symptom-free persons from the general population,[5] underlining the need to differentiate generally occurring bone erosions from disease associated bone erosions. A subsequent case-control study compared MRI-erosions of early RA-patients to MRI-erosions of symptom-free volunteers and patients with early arthritides other than RA. This study identified several erosion characteristics with a high specificity for RA as these almost never occurred in both reference groups; grade ≥2 erosions, erosions in metatarsophalangeal joint 5 (MTP5) and erosions in MTP1 in persons aged <40.[6]

With the ultimate aim to determine if the prognostic value of MRI could be improved by evaluating MRI-detected erosions, this study investigated if MRI-detected erosions are predictive for RA-development in patients with clinically suspect arthralgia (CSA) and if the prognostic accuracy of MRI could be improved by assessing MRI-detected erosions in addition to subclinical inflammation. We evaluated both the presence of any MRI-erosion and of MRI-erosion characteristics that were recently identified as RA-specific. Because it has been shown that erosions occur early in ACPA-positive patients in particular,[7-9] the analyses were stratified for ACPA.

Methods

Patients

Between April 2012-October 2018, 613 patients were included in the Leiden CSA cohort. CSA-patients had recent-onset (<1 year) arthralgia in the small joints, which was likely to progress to RA based on the clinical expertise of the rheumatologist. Per definition, patients were excluded if arthritis was detected upon physical examination or if a different explanation for the joint pain was more likely. Baseline visit consisted of physical examination, questionnaires, blood sampling and MRI. Follow-up visits were scheduled at 4, 12 and 24 months. When necessary, for instance in case of an increase of symptoms or when patients experienced joint swelling, additional visits were planned. Follow-up ended when patients developed arthritis, or else after 2-years. The cohort has been described in detail previously.[10]

All patients gave written informed consent. The study was approved by the local medical ethical committee.

MRI

Within two weeks after inclusion, CSA-patients underwent contrast-enhanced 1.5T MRI of wrist, 2nd-5th metacarpophalangeal (MCP) and 1st-5th MTP joints of the most painful side (in case of equally severe symptoms on both sides, the dominant side was scanned). For a detailed scanning protocol, see Supplementary File 1. Erosions, BME and synovitis were scored according to the RA MRI scoring system (RAMRIS),[11] tenosynovitis according to Haavardsholm.[12] Scoring was performed independently by two trained readers. Interreader and intrareader intraclass correlation coefficients were ≥0.91 and ≥0.92, respectively (Supplementary File 2).

MRI-erosion characteristics

Mean total erosion scores were studied, calculated by summation of mean erosion scores from both readers from all individual bones.

Next, as MRI-erosions also can be present in the general population, scores were dichotomized with MRI-erosion data of symptom-free controls as reference (n=193, as published previously).[5] Then patients were considered positive for MRI-erosions if ≥1 erosion that is uncommon in symptom-free controls, i.e. present in <5% of symptom-free controls in the same bone and in the same age category (<40, 40-59, ≥60), was present.

Lastly, erosion characteristics recently identified as RA-specific were evaluated; presence of grade ≥2 erosions, MTP5 erosions and MTP1 erosions when aged <40.

Outcome

The main outcome was development of inflammatory arthritis, determined by the rheumatologist at physical examination (66 swollen joint count ≥1). The secondary outcome was RA-development (fulfilment of 1987- or 2010-criteria).[13,14]

During follow-up (and before the main outcome was reached) treatment with disease-modifying antirheumatic drugs (DMARDs) (including steroids) was not allowed. Since April 2015, CSA-patients with MRI-detected subclinical inflammation could participate in a randomized double-blind placebo-controlled trial (RCT; TREAT EARLIER), studying the effect of Methotrexate in preventing RA-development. This RCT is still ongoing; patients enrolled in this trial (n=89) were excluded from the present study because of their 50% chance of DMARD-use.

Statistics

Total erosion scores and prevalence of MRI-erosions were evaluated with Mann-Whitney U and χ^2 tests. Cox proportional hazards regression was used to investigate predictive value. Multivariable models were adjusted for age and presence of MRI-detected subclinical inflammation (defined as synovitis, tenosynovitis and/or BME present in <5% of symptom-free controls in the same bone and in the same age category). Here all follow-up data was used. Analyses were stratified for ACPA. After 1-year follow the area under the curve (AUC) and the net reclassification index (NRI; the added value of MRI-detected erosions to subclinical inflammation) were determined.

Three subanalyses were performed. First, subanalyses were performed with the secondary outcome RA-development. Secondly, analyses were performed in the subgroup of CSA-patients that fulfilled the EULAR-definition of arthralgia suspicious for progression to RA (≥3/7 items present),[15] to study results in a more homogeneous CSA-population. Lastly, analyses were performed in patients included between April 2012-April 2015, i.e. before the start of the RCT, to investigate if excluding patients with subclinical inflammation affected the results.

P-values <0.05 were considered statistically significant. IBM SPSS Statistics Version 23 was used.

Results

Patients

Of 613 included patients, 123 were excluded (no MRI, participation in RCT; Supplementary File 3). Baseline characteristics are shown in Supplementary Table 1. 83 patients developed inflammatory arthritis after a median follow-up of 14 weeks (IQR 3-23). The median follow-up duration of patients that did not progress to inflammatory arthritis (n=407) was 103 weeks (IQR 51-113).

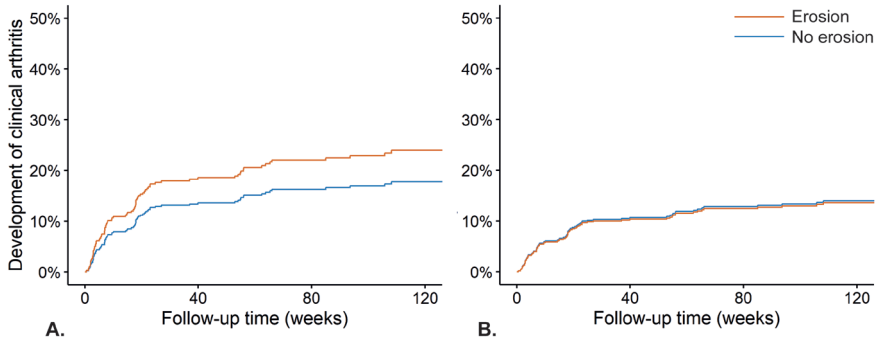
Total erosion scores and arthritis development

The median total erosion score in patients who progressed to inflammatory arthritis was 1.5 versus 1.0 in patients that did not progress. Erosion scores were associated with arthritis development in univariable analysis (HR 1.12 (95% CI 1.01-1.23)), but not after adjustments for age and subclinical inflammation (HR 0.97 (0.85-1.10)) (Table 1).

Presence of MRI-erosion and arthritis development

Next, only those erosions present in <5% of the general population in the same bone and age category were considered. These MRI-erosions were present in 20% of CSA-patients. In 60% of these patients subclinical inflammation was also present, in 40% there was no subclinical inflammation. Presence of MRI-detected erosions was not associated with arthritis development in univariable (HR 1.40 (0.86-2.28)) and multivariable analysis adjusted for age and subclinical inflammation (HR 0.97 (0.59-1.59)) (Table 1, Figure 1).

Figure 1. Development of inflammatory arthritis in presence/absence of erosions in univariable (A) and multivariable (B) analyses



Erosions were considered present if the MRI-erosion was uncommon in symptom-free controls, i.e. present in <5% of symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60). Multivariable models were adjusted for presence of subclinical inflammation. The HR (95% CI) for univariable and multivariable analyses were 1.40 (0.86-2.28) and 0.97 (0.59-1.59), respectively. HR: hazard ratio, CI: confidence interval

Table 1. Erosion scores, prevalence and association with development of inflammatory arthritis in patients with CSA

	No arthritis (n=407)	Arthritis (n=83)	Univariable Cox regression		Multivariable Cox regression[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.0 (0.5-2.5)	1.5 (0.5-3.5)	1.12 (1.01-1.23)	0.033	0.97 (0.85-1.10)	0.61
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	78 (19.2)	22 (26.5)	1.40 (0.86-2.28)	0.18	0.97 (0.59-1.59)[b]	0.90
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	7 (1.7)	3 (3.6)	1.84 (0.58-5.84)	0.30	1.05 (0.33-3.34)	0.94
MTP5 erosion	23 (5.7)	6 (7.2)	1.28 (0.56-2.95)	0.56	1.08 (0.47-2.48)	0.86
MTP1 erosion if age <40 (n=192)	11 (6.7)	2 (7.4)	1.16 (0.28-4.92)	0.84	1.11 (0.26-4.70)[b]	0.89

[a] Adjusted for age and presence of subclinical inflammation
[b] Adjusted for presence of subclinical inflammation
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint

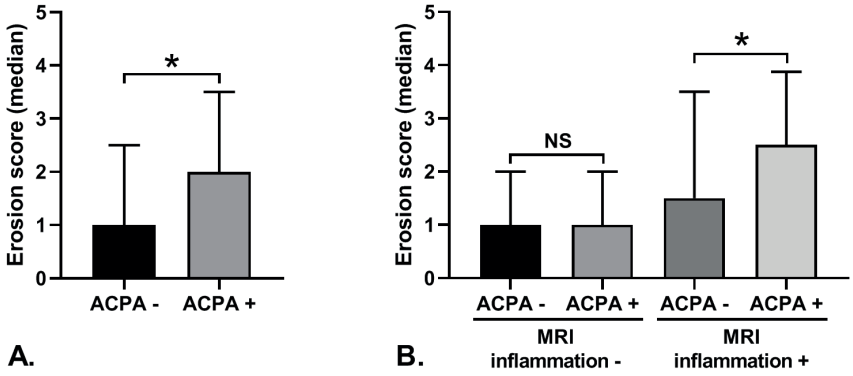
RA-specific erosion characteristics and arthritis development

Subsequently we studied the predictive value of erosion characteristics previously defined as RA-specific. Grade ≥2 erosions, MTP5 erosions, and MTP1 erosions in patients aged <40 were not associated with progression to inflammatory arthritis (multivariable HR 1.05 (0.33-3.34), 1.08 (0.47-2.48) and 1.11 (0.26-4.70), respectively) (Table 1).

Analyses of ACPA-positive and ACPA-negative patients

As ACPA-positive and ACPA-negative RA are different subsets, analyses were stratified for ACPA. ACPA-positive patients had significantly higher erosion scores than ACPA-negative patients (median 2.0 versus 1.0, p=0.002) (Figure 2A). However, when subclinical inflammation was also considered, this difference was only seen in patients with subclinical inflammation but not in ACPA-positive CSA-patients without subclinical inflammation (Figure 2B). Thus presence of ACPA without inflammation did not result in a higher erosion-score.

Figure 2. Erosion scores in ACPA-positive and ACPA-negative patients with and without concurrent subclinical inflammation



Graphs show total erosion scores in ACPA-positive and ACPA-negative patients (A), also stratified for presence of MRI-detected subclinical inflammation (B). *p<0.05, NS: non-significant, ACPA: anti-citrullinated protein antibody

Subsequently the predictive value of presence of MRI-erosions was assessed within each ACPA subset, and neither presence of any MRI-erosion, nor RA-specific erosions, were predictive for arthritis development in univariable and multivariable analyses (Supplementary Table 2 and 3).

Prognostic accuracy of MRI-erosions when added to MRI-inflammation

After 1-year follow-up (n=434) the AUC of any MRI-erosion to predict inflammatory arthritis development was 0.54. For comparison, the AUC of MRI-detected subclinical inflammation was 0.73. The AUC of both erosions and subclinical inflammation was

also 0.73. To determine if MRI-erosions improved the prognostic accuracy, the NRI was also determined. When erosion-data was added to the presence of subclinical inflammation, 35 patients (8.1%) were reclassified, 2 correctly, 33 incorrectly. This resulted in an NRI of -5.8, revealing no improved prognostic accuracy. Thus, the prognostic accuracy of MRI-detected subclinical inflammation did not improve, but in fact created a high number of false-positive predictions, when MRI-detected erosions were also assessed.

Subanalyses

MRI-erosions were not predictive with the outcome RA-development (n=490), within CSA-patients that fulfilled the EULAR-definition (n=317), and in patients included before the start of the RCT (n=225) (Supplementary Table 4-6).

Discussion

This study investigated if MRI-detected erosions in CSA-patients are predictive for development of inflammatory arthritis or RA. No association was found and MRI-detected erosions did not improve prognostic accuracy of MRI-detected subclinical inflammation. This implies that evaluating MRI-erosions of CSA-patients is superfluous if MRI-detected subclinical inflammation is assessed.

Until now the predictive value of MRI-detected erosions in CSA has not been studied longitudinally. A recent longitudinal study in patients presenting with undifferentiated arthritis (UA) showed that also in these patients MRI-erosions were not predictive for RA-development.[16] Interestingly, frequencies of any MRI-erosion or RA-specific erosions found in UA were quite similar as currently observed in CSA. Although we did not determine the frequency of presence of any MRI-erosions during IA-development, the finding of similar prevalence in UA and CSA suggests that the frequency of erosions did not increase over time. This would be in line with results from a previous study showing that the total MRI-erosion score did not increase during progression from CSA to RA.[17] Most importantly, the data together demonstrate that MRI-erosions in CSA and UA are not predictive for progression to the disease stage of RA. This result is different from previous findings on radiographic erosions in early RA, that are highly predictive for further radiographic progression.

Previous studies identified 'RA-specific erosions' by comparing patients with RA with other early arthritides. The present study revealed that 'RA-specific erosions' (that were identified in the phase of clinically apparent arthritis) are infrequent in the phase CSA and not prognostically valuable.

Even though MRI-detected erosions were not associated with RA-development, higher erosion scores were present in ACPA-positive compared to ACPA-negative patients; which is similar to our previous finding, done in the same cohort.[7] In our view these data suggest that presence of subclinical inflammation in ACPA-positive arthralgia is mediating the development of erosions. Whether ACPA can directly induce erosions, without an intermediary effect of inflammation, remains questionable and our data could not find support for this notion. Furthermore, this study added novel data to the field by demonstrating that MRI-erosions were not associated with progression to RA within ACPA-positive CSA-patients or within ACPA-negative CSA-patients.

Mouse models have suggested that osteoclast formation occurs early in the preclinical phase and before the development of inflammatory arthritis.[18] In the present cohort, of the CSA-patients with erosions (20%), 40% had no concomitant subclinical inflammation. Interestingly, this concerned both ACPA-positive and ACPA-negative patients (Figure 2). It can be speculated that erosions in these patients were the result of preceding subclinical inflammation. However, in absence of subclinical inflammation, RA-development was low.[3] This suggests that the presence of grade 1 MRI-detected erosions, without subclinical inflammation, is often not a feature of imminent RA. Perhaps additional stimuli needed for progression were lacking.

Since April 2015, CSA-patients with MRI-detected subclinical inflammation could participate in an RCT studying Methotrexate. Patients that entered this trial were excluded from analyses (Supplementary File 3). The group of patients in the present study that was included after April 2015 had less often subclinical inflammation than patients included before April 2015 (33% versus 51%); demonstrating that part of the patients with subclinical inflammation, a risk factor for arthritis development, was excluded. This might have resulted in over- or underestimation of the association between erosions and arthritis development. Although the frequency of subclinical inflammation was lower since the start of the RCT, the ratio of erosion presence within strata of patients with or without subclinical inflammation generally remains unchanged. Additionally, known risk factors for arthritis development were comparable for patients with subclinical inflammation who did and did not participate in the RCT. Hence, a possible influence on the effect in the total cohort can be eliminated by stratifying for subclinical inflammation; also then MRI-erosions were not predictive (Supplementary Table 7 and 8). Furthermore, subanalyses evaluating only patients included before April 2015, revealed similar results. Therefore we consider it unlikely that exclusion of patients because of the RCT caused false-negative results.

In conclusion, this large longitudinal study showed that MRI-detected erosions in

hands and feet of patients with CSA are not predictive for arthritis development. Therefore, evaluating MRI for erosions in addition to subclinical inflammation does not provide added prognostic value in CSA.

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Supplementary File 1 – MRI scanning and scoring protocol

Detailed MRI scan protocol

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

In the hand (metacarpophalangeal (MCP) joints 2-5 and wrist) the following sequence was acquired before contrast administration: T1-weighted fast spin-echo (FSE) sequence in the coronal plane (repetition time (TR) 575 ms, echo time (TE) 11.2 ms, acquisition matrix 388×288, echo train length (ETL) 2). After intravenous injection of gadolinium contrast (gadoteric acid, Guerbet, Paris, France, standard dose of 0.1 mmol/kg) the following sequences were obtained: T1-weighted FSE sequence with frequency selective fat saturation (fatsat) in the coronal plane (TR/TE 700/9.7ms, acquisition matrix 364×224, ETL 2), T1-weighted FSE fatsat sequence in the axial plane (wrist: TR/TE 540/7.7 ms; acquisition matrix 320x192; ETL 2 and MCP-joints: TR/TE 570/7.7 ms; acquisition matrix 320x192; ETL 2).

The obtained sequences of the forefoot (metatarsophalangeal (MTP) joints 1-5) were for the first 77 patients before contrast administration: T1-weighted FSE sequence in the axial plane (TR/TE 650/17ms; acquisition matrix 388x288, ETL 2); and T2-weighted FSE fatsat sequence in the axial plane (TR/TE 3000/61.8; acquisition matrix 300x224, ETL 7). Imaging of the foot was initially limited to pre-contrast axial sequences. For the latter 413 patients post-contrast sequences were included: T1-weighted FSE fatsat sequence in the axial plane (TR/TE 700/9.5ms; acquisition matrix 364x224, ETL 2) and: T1-weighted FSE fatsat sequence in the coronal plane (perpendicular to the axis of the metatarsals) (TR/TE 540/7.5ms; acquisition matrix 320x192, ETL 2).

Field-of-view was 100mm for the hand and 140mm for the foot. Coronal sequences of the hand had 18 slices with a slice thickness of 2mm and a slice gap of 0.2mm. Coronal sequences of the foot had 20 slices with a slice thickness of 3mm and a slice gap of 0.3mm. All axial sequences had a slice thickness of 3mm and a slice gap of 0.3mm with 20 slices for the wrist, 16 for the metacarpophalangeal-joints and 14 for the foot.

We used the contrast enhanced T1-weighted fat suppressed sequence to assess BME in the MCP-joints of all patients. In the MTP-joints BME was assessed on T2-weighted fatsat sequences in the first 77 patients and on the contrast enhanced T1-weighted

fat suppressed sequence in the latter patients. According to 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, three previous studies have demonstrated that a contrast enhanced T1-weigthed fat suppressed sequence has a strong correlation with T2-weighted fat suppressed sequences.[1-3] Furthermore, the arthritis subcommittee of the European Society of Musculoskeletal Radiology (ESSR) also recommends the use of contrast enhanced T1-weighted fat suppressed sequences for depicting BME.[4] The T2-weighted image shows increased water signal and a contrast-enhanced T1-weighted sequence shows increased water content and the increased perfusion and interstitial leakage. A strong correlation has been shown in arthritis patients and in patients without inflammatory diseases such as bone bruises, intraosseous ganglions, bone infarcts and even nonspecific cases.[2,3] Based on these results BME was assessed on contrast enhanced T1-weighted fat suppressed sequences as it has a higher signal to noise ratio and allowed a shorter scan time for patients. In addition, because T2-weighted fat suppressed sequences could be omitted, coronal sequences of the foot could be added. In total this resulted in a shorter total scan time and more information.

MRI scoring

All bones, joints and tendons were scored semi-quantitatively according to the validated RA MRI scoring system (RAMRIS). 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.). BME was scored on a scale 0-3 based on the affected volume of the bone (no BME, >0-33%, >33-66%, >66%) and synovitis was scored on a range 0-3 based on the volume of enhancing tissue in the synovial compartment (none, mild, moderate, severe).[5] Similar to methods described by Haavardsholm et al the tenosynovitis-score was based on the thickness of peritendinous effusion or synovial proliferation with contrast enhancement (normal, <2mm, 2-5mm, >5mm (range 0-3)).[6]

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Supplementary File 2 – Inter- and intrareader correlation

MRI scans of CSA-patients and symptom-free controls were scored by two readers according to the RAMRIS. A total of nine readers was available and different combinations of readers were used. All readers were trained in the same way, and interreader intraclass correlation coefficients (ICC) were ≥0.91. All intrareader ICCs were ≥0.92. See the Tables below for an overview of all ICC values.

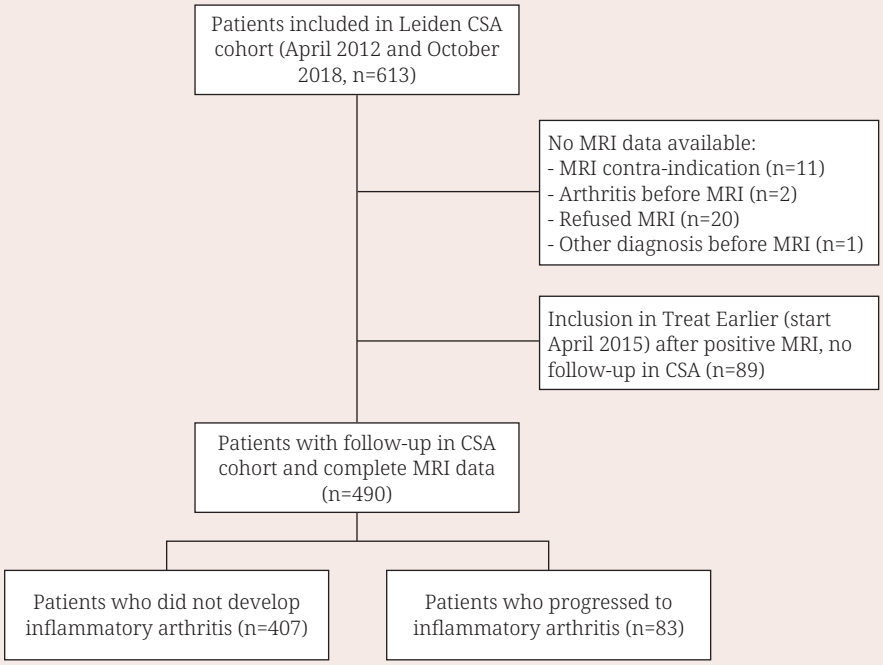
Interreader intraclass correlation coefficients

	1	2	3	4	5	6	7	8	9
1	x	0.97	0.97	0.98	0.97	0.96	0.95	0.97	0.93
2	0.97	x	0.99	0.95	0.94	0.95	0.94	0.96	0.93
3	0.97	0.99	x	0.95	0.95	0.95	0.96	0.96	0.94
4	0.98	0.95	0.95	x	0.97	0.96	0.94	0.95	0.91
5	0.97	0.94	0.95	0.97	x	0.95	0.94	0.95	0.92
6	0.97	0.95	0.95	0.96	0.95	x	0.95	0.96	0.95
7	0.95	0.94	0.96	0.94	0.94	0.95	x	0.98	0.98
8	0.97	0.96	0.96	0.95	0.95	0.96	0.98	x	0.96
9	0.93	0.93	0.94	0.91	0.92	0.95	0.98	0.96	x

Intrareader intraclass correlation coefficients

1	2	3	4	5	6	7	8	9
0.99	0.98	0.94	0.92	0.96	0.94	0.98	0.99	0.96

Supplementary File 3 – Patient selection flowchart



Patients that progressed to inflammatory arthritis had a median follow-up of 14 weeks (IQR 3-23). The median follow-up duration of patients that did not progress was 103 weeks (IQR 51-113).

Supplementary Table 1. Baseline characteristics of CSA patients

	All CSA patients (n=490)	ACPA negative (n=425)	ACPA positive (n=65)
Age in years, mean (SD)	43.6 (12.7)	43.0 (12.6)	47.6 (12.7)
Female, n (%)	379 (77.3)	326 (76.7)	53 (81.5)
Symptom duration in weeks, median (IQR)	19 (9-43)	18 (9-41)	22 (13-53)
68-TJC, median (IQR)	5 (2-10)	5 (2-11)	3 (2-7)
ACPA positivity (≥7 U/mL), n (%)	65 (13.3)	NA	NA
RF positivity (≥3.5 IU/mL), n (%)	97 (19.8)	47 (11.1)	50 (76.9)
Increased CRP (≥5 mg/L), n (%)	98 (21.1)	78 (19.3)	20 (33.3)
Presence of local subclinical inflammation[a], n (%)	202 (41.2)	154 (36.2)	48 (73.8)

[a] Presence of MRI-detected subclinical inflammation that is uncommon in symptom-free controls, i.e. present in <5% of the symptom-free controls at the same location and in the same age category (<40, 40-59, ≥60).
CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, RF: rheumatoid factor, CRP: c-reactive protein, NA: not applicable

Supplementary Table 2. Erosion scores, prevalence and association with development of inflammatory arthritis in ACPA-negative patients with CSA

	No arthritis (n=378)	**Arthritis (n=47)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.0 (0.5-2.0)	1.5 (0.5-3.0)	1.08 (0.93-1.24)	0.33	0.95 (0.79-1.14)	0.59
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	70 (18.5)	9 (19.1)	0.98 (0.48-2.03)	0.96	0.72 (0.35-1.50)[b]	0.38
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	7 (1.9)	0 (0.0)	0.048 (0.00-416)	0.51	NA	NA
MTP5 erosion	20 (5.3)	3 (6.4)	1.19 (0.37-3.83)	0.77	1.12 (0.35-3.63)	0.85
MTP1 erosion if age <40 (n=173)	10 (6.4)	1 (6.3)	1.05 (0.14-7.97)	0.96	0.99 (0.13-7.53)[b]	0.99

[a] Adjusted for age and presence of subclinical inflammation
[b] Adjusted for presence of subclinical inflammation
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint, ACPA: anti-citrullinated protein antibody, NA: not applicable

Supplementary Table 3. Erosion scores, prevalence and association with development of inflammatory arthritis in ACPA-positive patients with CSA

	No arthritis (n=29)	**Arthritis (n=36)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	2.0 (0.5-3.5)	2.3 (0.8-3.5)	1.01 (0.88-1.17)	0.84	0.93 (0.79-1.10)	0.39
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	8 (27.6)	13 (36.1)	1.16 (0.59-2.29)	0.67	0.91 (0.46-1.81)[b]	0.78
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	0 (0.0)	3 (8.3)	3.26 (0.97-10.95)	0.056	2.91 (0.86-9.83)	0.085
MTP5 erosion	3 (10.3)	3 (8.3)	0.93 (0.28-3.02)	0.90	0.62 (0.18-2.10)	0.45
MTP1 erosion if age <40 (n=19)	1 (12.5)	1 (9.1)	0.86 (0.11-6.74)	0.88	2.16 (0.23-20.12)[b]	0.50

[a] Adjusted for age and presence of subclinical inflammation
[b] Adjusted for presence of subclinical inflammation
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint, ACPA: anti-citrullinated protein antibody

Supplementary Table 4. Erosion scores, prevalence and association with RA-development (1987- or 2010-criteria) in patients with CSA

	No RA (n=430)	**RA (n=60)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.0 (0.5-2.5)	2.0 (0.5-3.5)	1.13 (1.00-1.27)	0.042	0.94 (0.81-1.09)	0.39
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	83 (19.3)	17 (28.3)	1.54 (0.88-2.69)	0.13	1.03 (0.58-1.82)[b]	0.92
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	8 (1.9)	2 (3.3)	1.71 (0.42-6.99)	0.46	0.89 (0.22-3.69)	0.88
MTP5 erosion	24 (5.6)	5 (8.3)	1.50 (0.60-3.74)	0.39	1.23 (0.49-3.07)	0.66
MTP1 erosion if age <40 (n=192)	12 (6.9)	1 (5.9)	0.91 (0.12-6.86)	0.93	0.84 (0.11-6.37)[b]	0.87

[a] Adjusted for age and presence of subclinical inflammation
[b] Adjusted for presence of subclinical inflammation
RA: rheumatoid arthritis, CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, MTP: metatarsophalangeal joint

Supplementary Table 5. Erosion scores, prevalence and association with development of inflammatory arthritis in CSA-patients fulfilling EULAR-definition[a]

	No arthritis (n=257)	**Arthritis (n=60)**	**Univariable Cox regression**		**Multivariable Cox regression[b]**	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.0 (0.5-2.5)	1.5 (0.5-3.5)	1.09 (0.96-1.25)	0.18	0.96 (0.82-1.13)	0.61
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	53 (20.6)	16 (26.7)	1.27 (0.72-2.25)	0.41	0.92 (0.52-1.64)[c]	0.78
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	6 (2.3)	1 (1.7)	0.69 (0.095-4.98)	0.71	0.45 (0.062-3.27)	0.43
MTP5 erosion	15 (5.8)	6 (10.0)	1.65 (0.71-3.84)	0.25	1.52 (0.65-3.54)	0.33
MTP1 erosion if age <40 (n=135)	8 (7.1)	1 (4.5)	0.70 (0.094-5.19)	0.73	0.57 (0.076-4.21)[c]	0.58

[a] Patients fulfilled the EULAR-definition of arthralgia suspicious for progression to RA if ≥3/7 items were present
[b] Adjusted for age and presence of subclinical inflammation
[c] Adjusted for presence of subclinical inflammation
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint

Supplementary Table 6. Erosion scores, prevalence and association with development of inflammatory arthritis in CSA-patients included before 2015

	No arthritis (n=182)	**Arthritis (n=43)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.5 (0.5-3.5)	1.5 (0.5-3.5)	1.01 (0.87-1.17)	0.92	0.93 (0.78-1.11)	0.42
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	49 (26.9)	12 (27.9)	1.01 (0.52-1.97)	0.98	0.72 (0.37-1.42)[b]	0.34
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	6 (3.3)	2 (4.7)	1.43 (0.34-5.90)	0.63	1.01 (0.24-4.24)	0.99
MTP5 erosion	15 (8.2)	4 (9.3)	1.15 (0.41-3.22)	0.79	1.06 (0.38-2.97)	0.91
MTP1 erosion if age <40 (n=84)	5 (7.2)	0 (0.0)	0.045 (0.00-391)	0.50	NA	NA

[a] Adjusted for age and presence of subclinical inflammation
[b] Adjusted for presence of subclinical inflammation
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint, NA: not applicable

Supplementary Table 7. Erosion scores, prevalence and association with development of inflammatory arthritis in CSA-patients without subclinical inflammation

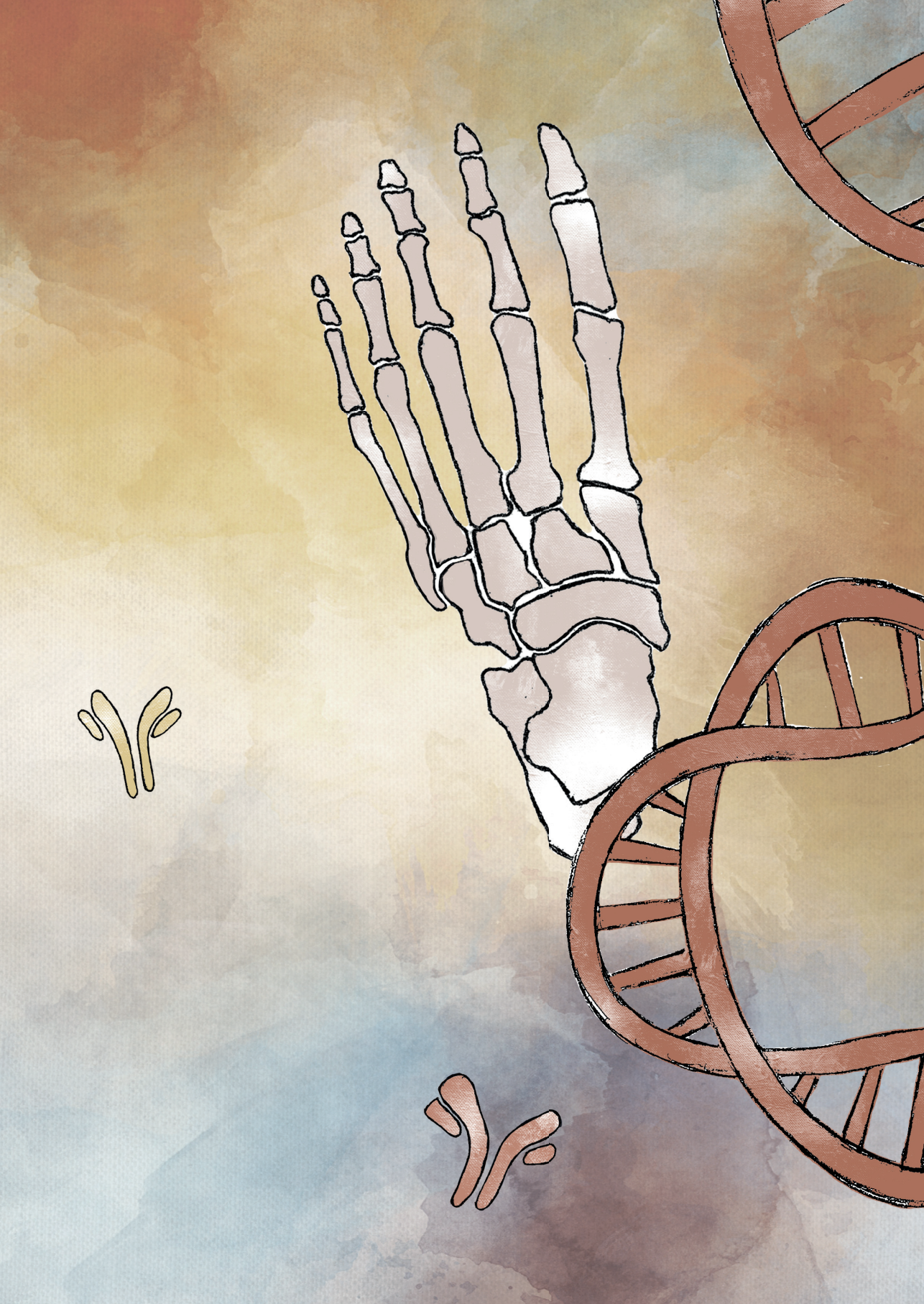
	No arthritis (n=271)	**Arthritis (n=17)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	1.0 (0.0-2.0)	1.0 (0.5-2.0)	1.01 (0.76-1.34)	0.96	1.04 (0.76-1.14)	0.80
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	37 (13.7)	3 (17.6)	1.23 (0.35-4.30)	0.74	NA	NA
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	2 (0.7)	1 (5.9)	7.67 (1.01-58.09)	0.049	8.13 (1.06-62.37)	0.044
MTP5 erosion	11 (4.1)	2 (11.8)	2.28 (0.51-10.10)	0.28	2.31 (0.52-10.29)	0.27
MTP1 erosion if age <40 (n=120)	7 (6.3)	0 (0.0)	0.045 (0-14854)	0.63	NA	NA

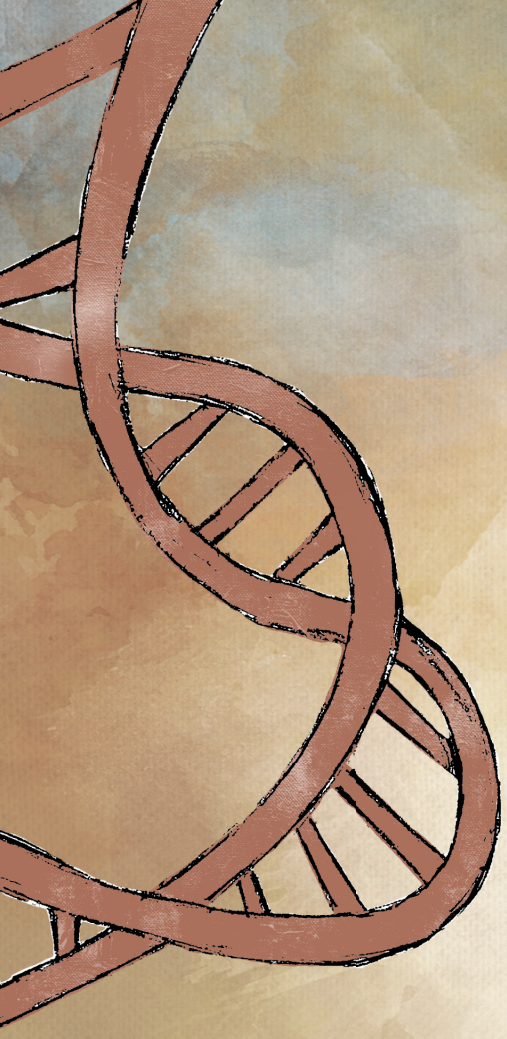
[a] Adjusted for age
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint, NA: not applicable

Supplementary Table 8. Erosion scores, prevalence and association with development of inflammatory arthritis in CSA-patients with subclinical inflammation

	No arthritis (n=136)	**Arthritis (n=66)**	**Univariable Cox regression**		**Multivariable Cox regression**[a]	
Continuous MRI-erosion data	*Erosion score, median (IQR)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Total erosion score	2.0 (0.5-3.5)	2.0 (1.0-3.5)	1.02 (0.91-1.15)	0.73	0.94 (0.82-1.09)	0.42
Dichotomized MRI-erosion data	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Presence of ≥1 erosion with symptom-free controls as reference	41 (30.1)	19 (28.8)	0.93 (0.54-1.58)	0.78	NA	NA
Erosion characteristics previously determined as RA-specific	*Prevalence, n (%)*		*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
Grade ≥2 erosion	5 (3.7)	2 (3.0)	0.79 (0.19-3.24)	0.75	0.71 (0.17-2.90)	0.63
MTP5 erosion	12 (8.8)	4 (6.1)	0.86 (0.31-2.36)	0.77	0.83 (0.30-2.29)	0.72
MTP1 erosion if age <40 (n=72)	4 (7.5)	2 (10.5)	1.52 (0.35-6.62)	0.57	NA	NA

[a] Adjusted for age
CSA: clinically suspect arthralgia, IQR: interquartile range, HR: hazard ratio, CI: confidence interval, RA: rheumatoid arthritis, MTP: metatarsophalangeal joint, NA: not applicable

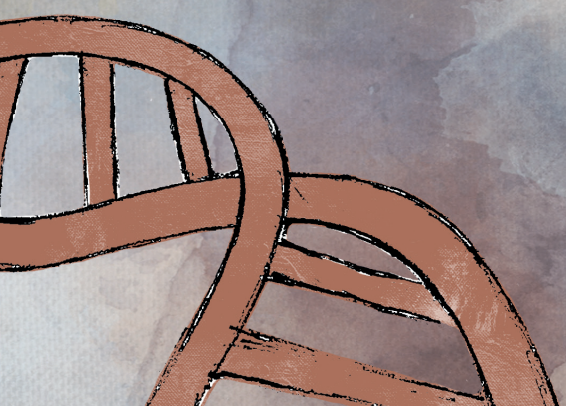


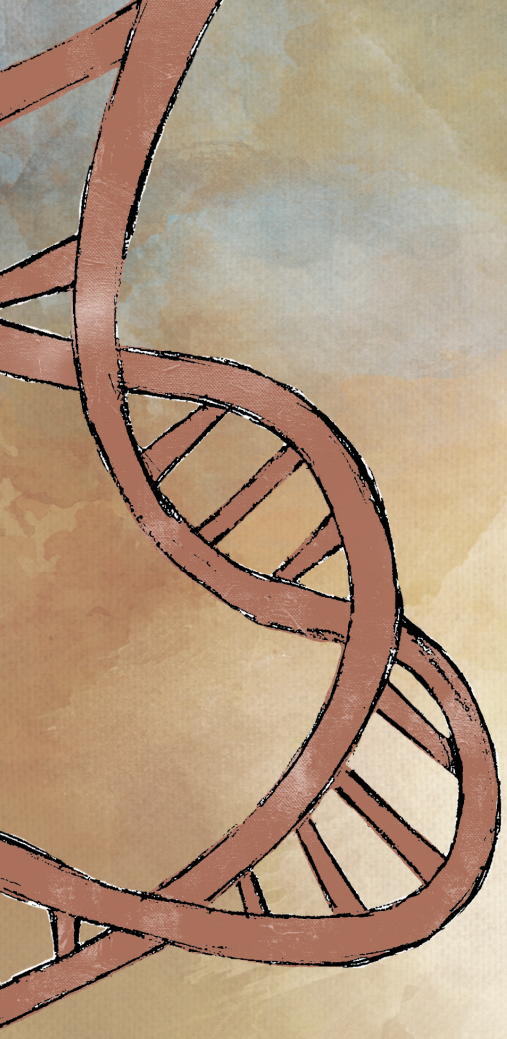


Pathogenesis of Rheumatoid Arthritis

Part

II





6

Do autoantibody-responses mature between presentation with arthralgia suspicious for progression to rheumatoid arthritis and development of clinically apparent inflammatory arthritis? A longitudinal serological study

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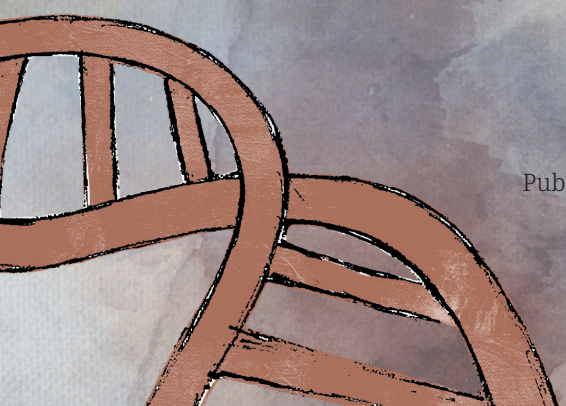

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Several nested case-control studies have shown that autoantibody-response maturation in rheumatoid arthritis (RA) precedes clinical arthritis-development.[1-3] This suggests a role in disease triggering. However, nested case-control studies have, similar to case-control studies, the disadvantage that controls are selected and that prospective data from non-progressing patients in a similar pre-disease stage are absent. The phase preceding clinically apparent inflammatory arthritis (IA) can be distinguished into an asymptomatic and symptomatic (i.e. clinically suspect arthralgia, CSA) sub-phase. It is unknown whether autoantibody-response maturation occurs in the symptomatic phase. Likewise, its role in progression to clinical arthritis is undetermined; if autoantibody-response maturation relates to disease-development, maturation is expected to be more pronounced in CSA-patients that progress compared to CSA-patients that do not. To better understand the relation between autoantibody-response maturation in time and development of clinical arthritis (RA/IA), we performed a longitudinal study on autoantibody-response maturation in CSA-patients that did and did not progress.

In serum from 147 CSA-patients, we determined with in-house ELISAs the presence and levels of IgM, IgG, IgA anti-citrullinated, anti-carbamylated and anti-acetylated protein antibodies (ACPA, anti-CarP, AAPA), resulting in 9 autoantibody measurements per patient per time-point. Autoantibody-response maturation was defined as increase in number of autoantibody-reactivities or isotypes, and/or increase in autoantibody levels. CSA-patients with paired samples at first presentation at the outpatient clinic and at IA-development (n=55) or else after 2-years (n=92) were selected. Analyses were repeated with the outcome RA (the subgroup of IA-patients that fulfilled the 2010-or 1987-criteria at the time of IA-development). Detailed description of methods and baseline characteristics are shown supplementary.

In patients negative for all autoantibodies at baseline, 17% of patients that progressed to IA became positive, compared to 6% of ”non-progressors” (Figure 1A, p=0.12). In patients with ≥1 autoantibody-reactivity at baseline progressing to IA, the median number of autoantibody-reactivities was 1.0 (IQR 1.0-3.5, max. 6) at baseline and 1.0 (IQR 1.0-4.0, max. 6) at IA-development (p=0.29). In non-progressing CSA-patients with ≥1 autoantibody-reactivity at baseline, this was 1.0 (IQR 1.0-2.0, max. 4) at baseline and 1.0 (IQR 0.0-2.3, max. 5) after 2-years (p=0.07). As shown in Figure 1B; an increase in the number of autoantibody-reactivities was infrequent (16% in progressors, 18% in non-progressors (p=1.00)). Most changes in autoantibody-positivity were explained by fluctuations around the cut-off (data not shown). Levels of autoantibodies did not significantly change over time (p-values ranging 0.21-1.00) both in progressors and non-progressors (Figure 1C). Similar results were found with the outcome RA (Supplementary Figure 1), though remarkably, the number of autoantibody-

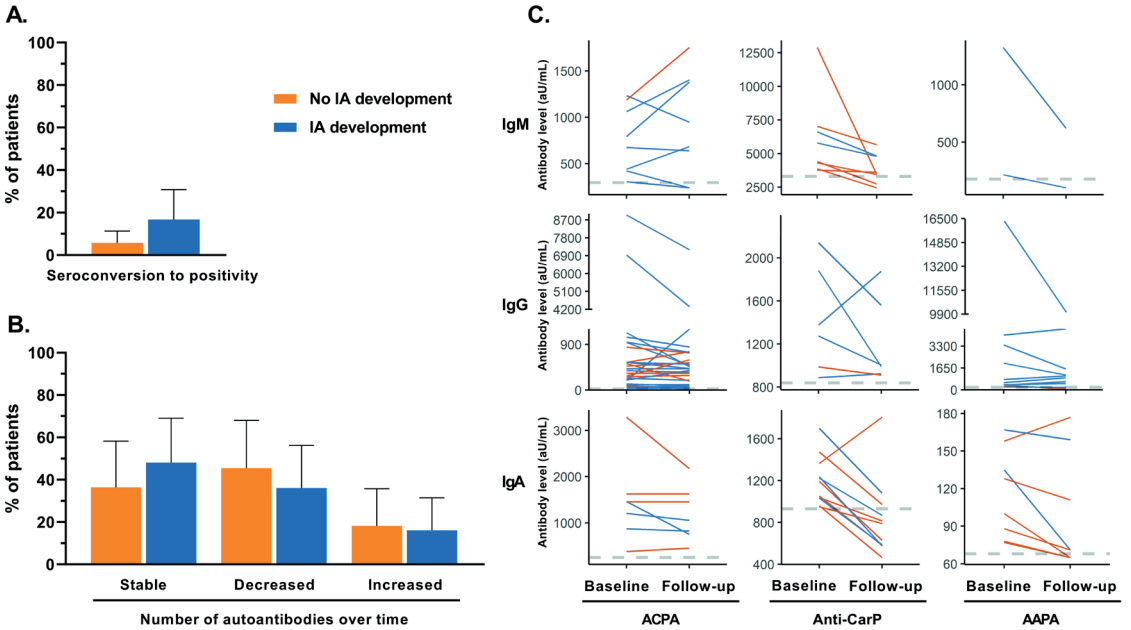
reactivities in patients not-progressing to RA significantly decreased over time (1.0 (IQR 1.0-2.0) at baseline and 1.0 (IQR 0.0-2.0) after 2-years, p=0.015). Finally, when evaluating number of autoantibody-reactivities and autoantibody-level changes within the entire study population (instead of within patients with ≥1 autoantibody-reactivity at baseline) no significant increases were found (Supplementary Figure 2).

To the best of our knowledge, this is the first study evaluating multiple isotypes and three anti-modified protein autoantibodies over time in CSA. Our data indicate that the presence and levels of IgM, IgG and IgA ACPA, anti-CarP and AAPA did not significantly increase over time, and that this was similar for CSA-patients that did or did not develop IA.

Autoantibody maturation in terms of cross-reactivity, affinity maturation and involvement of individual B-cell clones was not studied here, which is a limitation. We did not observe changes in isotype-usage over time, indicating that isotype switching was infrequent in both groups (Supplementary Figure 3, Supplementary Table 4). Although we cannot exclude that the results of this study would be different with a larger sample size (especially in CSA-patients autoantibody-negative at first presentation), the current data suggests that autoantibody-response maturation already occurs before presenting with CSA and that it does not increase substantially during progression to IA. Our results on characteristics of the ACPA, anti-CarP and AAPA-response expand on previous longitudinal studies showing similar ACPA- and RF-levels,[4,5] and absence of change in the ACPA antigen-recognition repertoire in ACPA-positive arthralgia.[6] The data together imply that maturation occurs predominantly in the asymptomatic phase, a finding to be confirmed in population-based studies. Moreover, in relation to a multiple-hit model for RA-development, our data suggest that autoantibody-response maturation in the CSA-phase is not related to the “final hit” as maturation was similar in CSA-patients not developing RA. These results increase the comprehension of the pathogenesis of RA.

In conclusion, autoantibody-response maturation as measured in this study occurs in the vast majority of CSA-patients before presenting with symptoms and broadening of the autoantibody-response is not specific for progression from arthralgia to clinical arthritis.

Figure 1. Changes in autoantibody-response over time: A) percentage of patients with seroconversion to positive in patients negative for all autoantibodies at baseline, B) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in patients positive for ≥1 autoantibody-reactivity at baseline, C) autoantibody levels over time in patients positive for the respective autoantibody at baseline.



All results are shown separately for CSA-patients that did and did not progress to IA. The mean time between first presentation and IA development was 5.6 months (SD 9.2). In patients that did not progress the second serum sample was obtained after 2-years.
Figure 1A autoantibody negativity at baseline was defined as negative for the nine studied measurements (n=100), Figure 1B autoantibody positive was defined as at least one (out of nine) positive measurement at baseline (n=47).
Error bars in Figure 1A and 1B represent 95% CI. Dashed grey horizontal lines in Figure 1C indicate the cut-off values for each autoantibody.
IA: clinically apparent inflammatory arthritis, ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies.

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Supplementary File 1 – Detailed description of methods

Patients

Patients with recent-onset (<1 year) arthralgia of small joints and, according to the clinical expertise and pattern recognition of the rheumatologist a clinical suspicion for progression to RA, were included in the Leiden CSA-cohort. Autoantibody status was largely unknown at inclusion as (in line with Dutch guidelines) general practitioners in the area of Leiden are discouraged to perform autoantibody tests. Inclusion in the CSA-cohort was therefore predominantly based on history taking and physical examination. Patients were excluded if clinically apparent inflammatory arthritis was already present, or if a different explanation for the joint pain was more likely. The cohort is described in detail previously.[1] Patients were followed for at least 2 years on the development of clinically apparent inflammatory arthritis (IA) with scheduled research visits after 4, 12 and 24 months. Clinical follow-up visits took place at the scheduled visits and at additional visits (either in between or after the scheduled visits), as considered necessary by patients or rheumatologists. Serum samples were taken at baseline and when patients progressed to IA, or, when patients did not progress to IA after 2-years. Patient selection for the present study was first based on availability of paired samples and subsequently on the presence of autoantibodies at baseline. The latter was done because of limited laboratory capacity. Patients that were tested positive for RF (in house ELISA, cut-off >3.5 IU/mL) and/or ACPA (anti-CCP2, Phadia, Nieuwegein, the Netherlands, cut-off >7U/mL) during routine laboratory measurements at baseline and had paired serum samples were included (n=59, 29 progressing and 30 non-progressing patients). In addition, autoantibody-negative patients with paired samples that progressed to IA were included (n=26). Finally, from the large group of autoantibody-negative patients that did not progress to IA a random selection was made (n=62). Supplementary Table 2 suggests that selection of patients with paired samples from the total CSA-cohort did not induce substantial selection bias. Similarly, baseline characteristics of the randomly selected autoantibody-negative patients were similar to that of the patients that were not selected (Supplementary Table 3); suggesting that the selection is representative for this total group. Thus, the similarity in baseline characteristics from selected and non-selected patients implies that the selected group of patients (N=147 in total) is representative and suitable to study autoantibody characteristics over time. However the fact that not all but a selection of autoantibody-negative CSA-patients was assessed makes the current selection not suitable to determine the predictive accuracy of autoantibodies, which was also not the aim of this study.

Autoantibodies

In serum, we determined the presence and levels of anti-citrullinated, anti-carbamylated and anti-acetylated protein antibodies (ACPA, anti-CarP and AAPA, respectively); all three autoantibodies have been shown to be present in RA. ACPA and anti-CarP have been shown to be associated with progression and/or prediction of disease and have a specificity of 95-100% and 95%, respectively.[2-6] The specificity of AAPA IgG in patients with RA, compared to non-RA patients with persistent or resolving arthritis was 86% in a previous study.[7] Cross-reactivity between all three autoantibodies has been shown.[8] In this study, presence of ACPA, anti-CarP and AAPA was determined for three isotypes (IgM, IgG and IgA), resulting in 9 autoantibody measurements per patient per time-point. In-house ELISA was used for all measurements as described previously.[9] Briefly, plates were coated with citrullinated CCP2, carbamylated FCS and CCP1 acetylated lysine for measurements of ACPA, anti-CarP and AAPA, respectively. To determine background signal, plates were additionally coated with non-modified antigens (arginine CCP2, non-modified FCS and CCP1 norleucine, respectively). Serum samples were diluted 1:50 and incubated. After washing, plates were subsequently incubated with HRP-labeled goat-anti-human IgM (Millipore), rabbit-anti-human IgG (Dako) or goat-anti-human IgA (Novex). HRP-activity was visualized with ABTS and measurements were expressed in arbitrary units per milliliter (aU/mL). On every plate a dilution standard was included to determine the linear part of the curve; standards from all plates were used in the analyses. The fourth standard, which is expected to be in the middle (and therefore linear part) of the curve, is further diluted and additionally included as a reference sample. Serum of healthy subjects (n=199) was used to determine the cut-off of all autoantibody measurements, which was calculated as the mean plus two times the standard deviation of healthy subjects. When the background signal of non-modified antigens was >50% of the signal measured in modified proteins, the measurement was considered non-specific; non-specific measurements with values above the cut-off were considered negative. In case a sample reached the upper detection limit of the assay, the sample pair (two samples of the same individual but from different time points) was reanalyzed in a higher dilution (2 samples for ACPA IgG in 1:2000, 2 samples for ACPA IgA in 1:250, 6 samples for AAPA IgG with dilutions ranging 1:100-1:2000). Samples were measured single well and paired samples, thus two samples of the same individual but from different time points, were analyzed on the same plate. Inter-assay variation of in-house ELISAs was determined previously by reevaluation of ~10% of samples; measurements were highly correlated (Pearson's r ranges 0.88-0.99) and changes in positivity of the test were infrequent, see Supplementary Figure 4. Intra-assay variability was determined for ACPA and anti-CarP IgM, IgG and IgA by measurement of 3 samples 10 times. The mean coefficients of variation (CV, mean % (SD)) were: ACPA IgM 13.5 (15.0), IgG 8.7 (6.2), IgA 3.4 (1.2), anti-CarP IgM 5.6 (3.7), IgG

20.4 (6.8), IgA 4.2 (1.1). Of note, although not absolute at the monoclonal- or polyclonal level, cross-reactivity of ACPA towards other post translationally modified proteins have been conclusively shown in different studies,[8,10] and hence should be regarded as anti-modified protein antibody-reactivities.

Outcome

The primary outcome was development of IA, determined by physical examination of the rheumatologist (assessment of clinical joint swelling) during follow-up. DMARDs (including glucocorticoids) were not prescribed in patients with CSA. In patients that progressed to IA, the second sample was taken at IA-development. In patients that did not progress to IA serum samples were taken after 2 years (last scheduled follow-up visit with serum collection). Theoretically, IA-development could have occurred after this 2 years-visit in these patients. Reassuringly however, this did not occur during the period for which clinical follow-up data was available (median 29 months (IQR 20-46) after the scheduled 2-years visit). We also assume that patients would have visited our outpatient clinic in case of an increase in symptoms or suspected arthritis, and therefore that these data are all-encompassing, since our outpatient clinic is the only referral center in a healthcare region of approximately 400.000 inhabitants and patients (especially those participating to clinical studies) have very easy access to our outpatient clinic.

Analyses were repeated with "development of RA" as outcome, which was defined by fulfilment of the 1987 and/or 2010 classification criteria for RA at the time clinically apparent arthritis (IA) had presented.[11,12] The 1987-criteria were incorporated in this definition as autoantibody-negative patients require >10 involved joints in the 2010-criteria to be classified as RA.

Statistical analyses

Autoantibody-response maturation over time was defined as an increase in number of autoantibody-reactivities or isotypes, and/or an increase in autoantibody levels. To evaluate autoantibody-response maturation three analyses were performed, in patients that progressed to IA (n=55) and in patients that did not progress (n=92) separately. First, in patients negative for all nine measurements at baseline, we determined the frequency of conversion to seropositivity. Importantly when showing the results from the analyses of the different isotypes of ACPA, AAPA and anti-CarP, autoantibody negativity was defined as negativity for these nine isotypes at baseline (n=100). Second, in patients with at least one positive test at baseline (n=47), we studied autoantibody positivity over time by evaluating the median number of positive autoantibody-reactivities over time and the frequency that the number of positive measurements changed. Finally, we determined the change in autoantibody

levels over time, for all autoantibodies and isotypes separately. In these analyses we only included patients positive for the respective measurement at baseline, e.g. for evaluation of changes in IgG ACPA levels over time we only included patients that were positive for IgG ACPA at baseline. Frequencies and medians were reported. Statistical significance of frequencies was tested with Fisher's Exact test. The number of autoantibody-reactivities over time was tested with generalized estimating equations (GEE), taking into account that measurements over time and within one autoantibody type (ACPA, anti-CarP or AAPA) can be correlated. Changes in autoantibody levels over time were tested with Wilcoxon Signed Ranks tests with Bonferroni correction for multiple testing.

Subanalyses

Two additional analyses were performed. First, analyses were repeated with the outcome development of RA. Secondly, the number of autoantibody-reactivities and autoantibody levels over time were evaluated within the entire study population (instead of within the group of patients that were autoantibody positive at baseline).

IBM SPSS Statistics 25 was used for all analyses. P-values ≤0.05 were considered statistically significant.

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Supplementary Table 1. Baseline characteristics of the studied CSA patients that did and did not progress to clinically apparent inflammatory arthritis (IA)

		IA during follow-up (n=55)	No IA during follow-up (n=92)	p-value
Clinical characteristics				
Female, n (%)		40 (72.7)	73 (79.3)	0.42
Age in years, mean (SD)		46.4 (12.9)	45.5 (12.8)	0.60
Symptom duration in weeks, median (IQR)		21 (8-51)	17 (10-37)	1.00
68-TJC, median (IQR)		5 (3-9)	5 (2-11)	0.82
Morning stiffness ≥60 minutes, n (%)		22 (40.0)	23 (25.0)	0.066
Difficulties making a fist, n (%)		14 (25.9)	10 (11.0)	0.036
Family history of RA, n (%)		16 (29.6)	17 (19.1)	0.16
Routine laboratory measurements				
Increased CRP (≥5 mg/L), n (%)		16 (29.1)	19 (20.7)	0.32
RF IgM positivity (≥3.5 IU/mL), n (%)		26 (47.3)	25 (27.2)	0.019
ACPA IgG positivity (≥7.0 IU/mL), n (%)		22 (40.0)	12 (13.0)	<0.001
Presence of autoantibodies with in-house ELISA, n (%)				
ACPA	IgM	8 (14.5)	1 (1.1)	0.002
	IgG	20 (36.4)	9 (9.8)	<0.001
	IgA	3 (5.5)	4 (4.3)	1.00
Anti-CarP	IgM	2 (3.6)	6 (6.5)	0.71
	IgG	5 (9.1)	1 (1.1)	0.028
	IgA	4 (7.3)	7 (7.6)	1.00
AAPA	IgM	2 (3.6)	0 (0.0)	0.14
	IgG	10 (18.2)	1 (1.1)	<0.001
	IgA	2 (3.6)	7 (7.6)	0.48

SD: standard deviation, IQR: interquartile range, TJC: tender joint count, CRP: c-reactive protein, RF: rheumatoid factor, ACPA: anti-citrullinated protein antibody, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies

Supplementary Table 2. Baseline characteristics of all CSA-patients included between 2012 and 2016, stratified for patients with available paired serum samples and patients with only baseline samples available

	Paired samples available	Only baseline samples available	p-value
Female, n (%)	171 (78.4)	119 (77.8)	0.90
Age in years, mean (SD)	45.3 (12.8)	40.9 (11.8)	0.001
Symptom duration in weeks, median (IQR)	17 (9-39)	17 (8-33)	0.23
68-TJC, median (IQR)	5 (2-10)	6 (2-11)	0.81
Increased CRP (≥5 mg/L), n (%)	41 (18.8)	33 (21.7)	0.51
RF positivity* (≥3.5 IU/mL), n (%)	49 (22.5)	27 (17.6)	0.30
ACPA positivity* (≥7 U/mL), n (%)	31 (14.2)	16 (10.5)	0.34

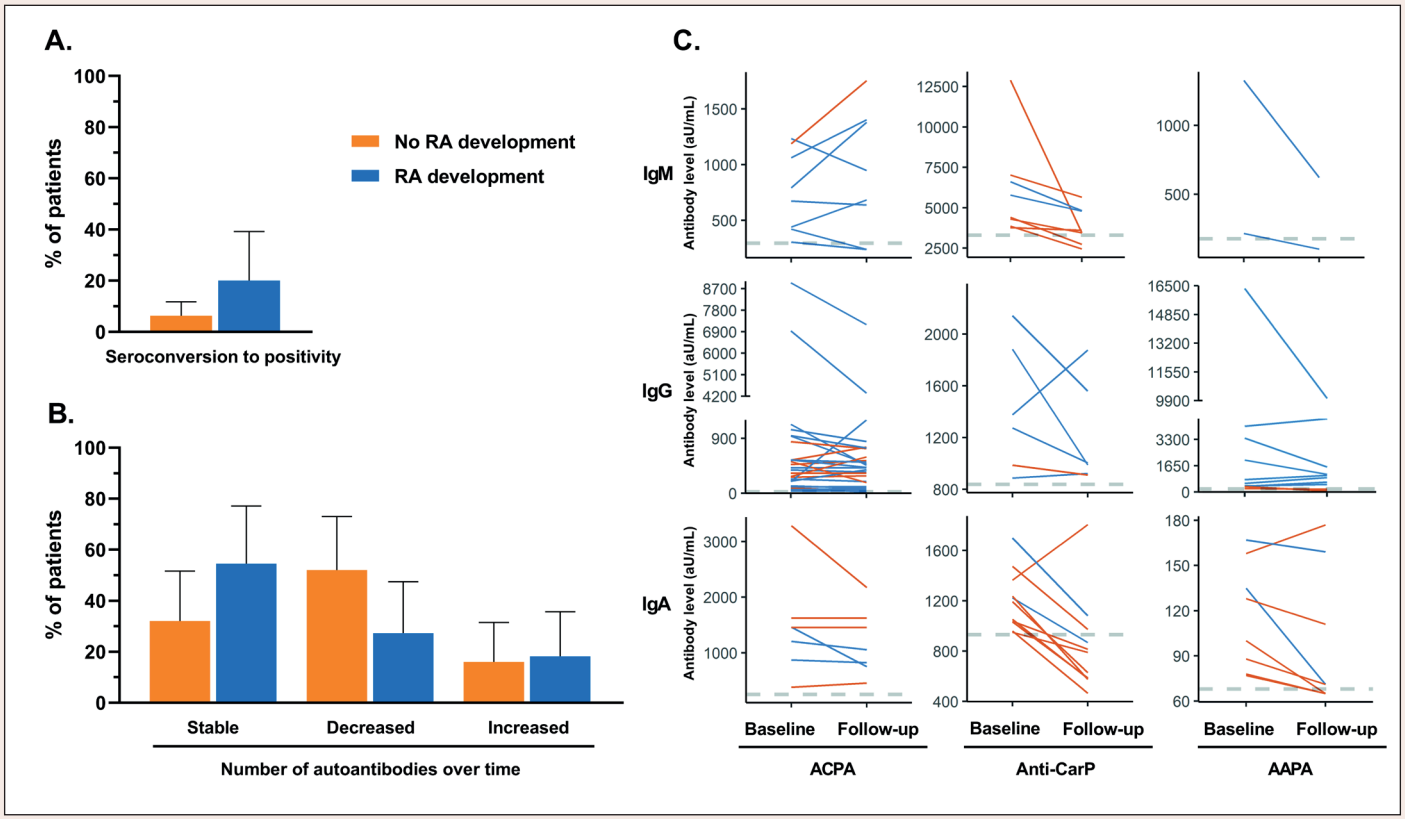
* based on routine laboratory diagnostics at baseline
CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, RF: rheumatoid factor, CRP: c-reactive protein

Supplementary Table 3. Baseline characteristics of the autoantibody-negative CSA-patients not progressing to IA with available paired samples that were randomly selected to be included and not included in this study

	Included based on random selection (n=62)	Not included based on random selection (n=77)	p-value
Female, n (%)	49 (79.0)	62 (80.5)	0.84
Age in years, mean (SD)	44.3 (13.6)	44.7 (12.7)	0.99
Symptom duration in weeks, median (IQR)	16 (9-29)	17 (9-45)	0.47
68-TJC, median (IQR)	7 (3-13)	6 (2-10)	0.55
Increased CRP (≥5 mg/L), n (%)	14 (22.6)	9 (11.7)	0.11
RF positivity* (≥3.5 IU/mL), n (%)	0 (0.0)	0 (0.0)	NA
ACPA positivity* (≥7 U/mL), n (%)	0 (0.0)	0 (0.0)	NA

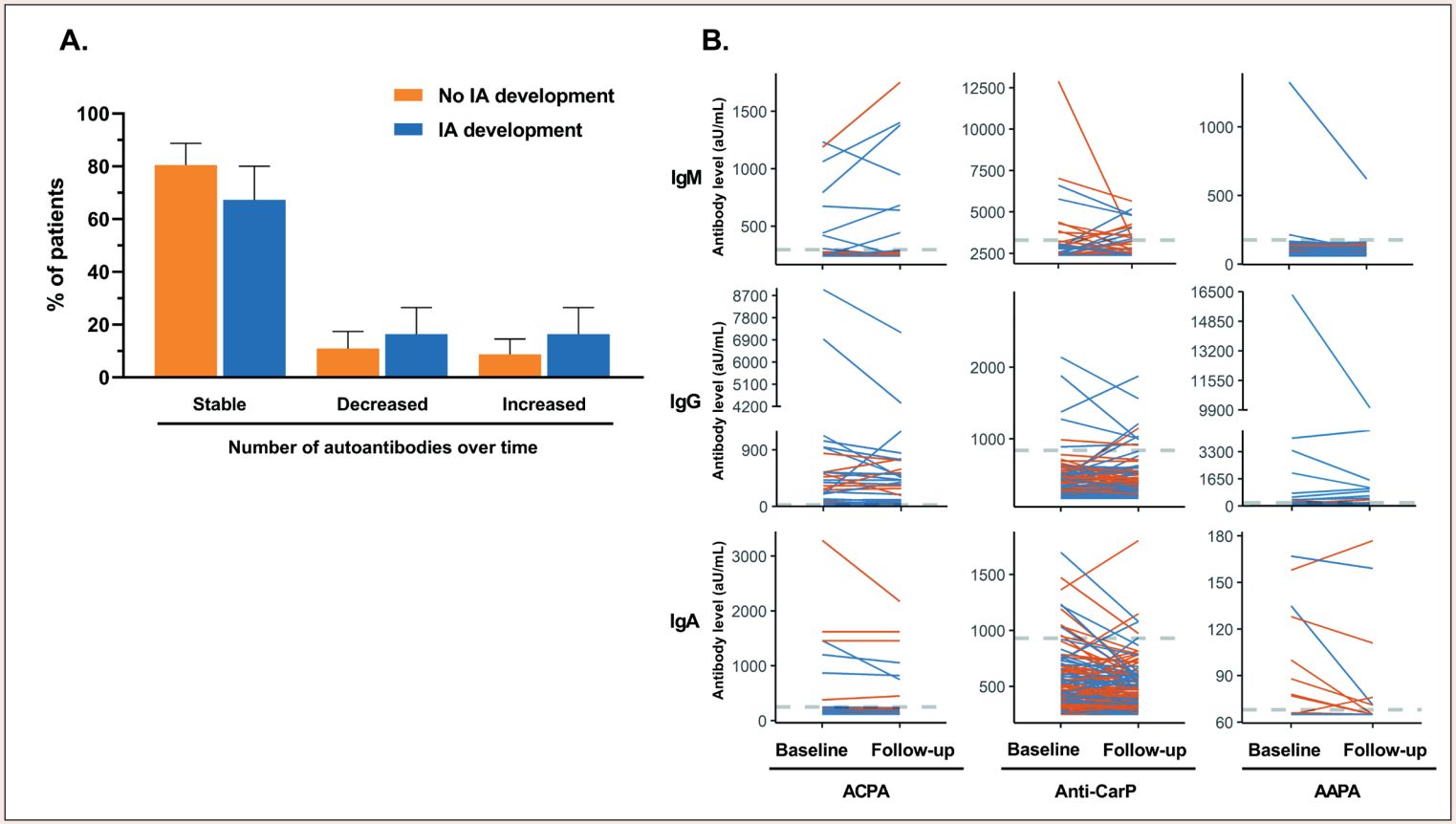
* based on routine laboratory diagnostics at baseline
The 62 RF and ACPA negative patients that did not progress and the 26 RF and ACPA negative patients that did progress to IA were selected for this study. Notably, for patient selection autoantibody negativity was defined as RF and ACPA negativity at baseline using routine diagnostics. When showing the results from the analyses of the different isotypes of ACPA, AAPA and anti-CarP in the manuscript, autoantibody-negativity was defined as negativity for the nine measured isotypes at baseline.
CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, RF: rheumatoid factor, CRP: c-reactive protein

Supplementary Figure 1. Changes in autoantibody-response over time in patients that did and did not progress to RA: A) percentage of patients with seroconversion to positive in patients negative for all autoantibodies at baseline, B) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in patients positive for ≥1 autoantibody-reactivity at baseline, C) autoantibody levels over time in patients positive for the respective autoantibody at baseline.



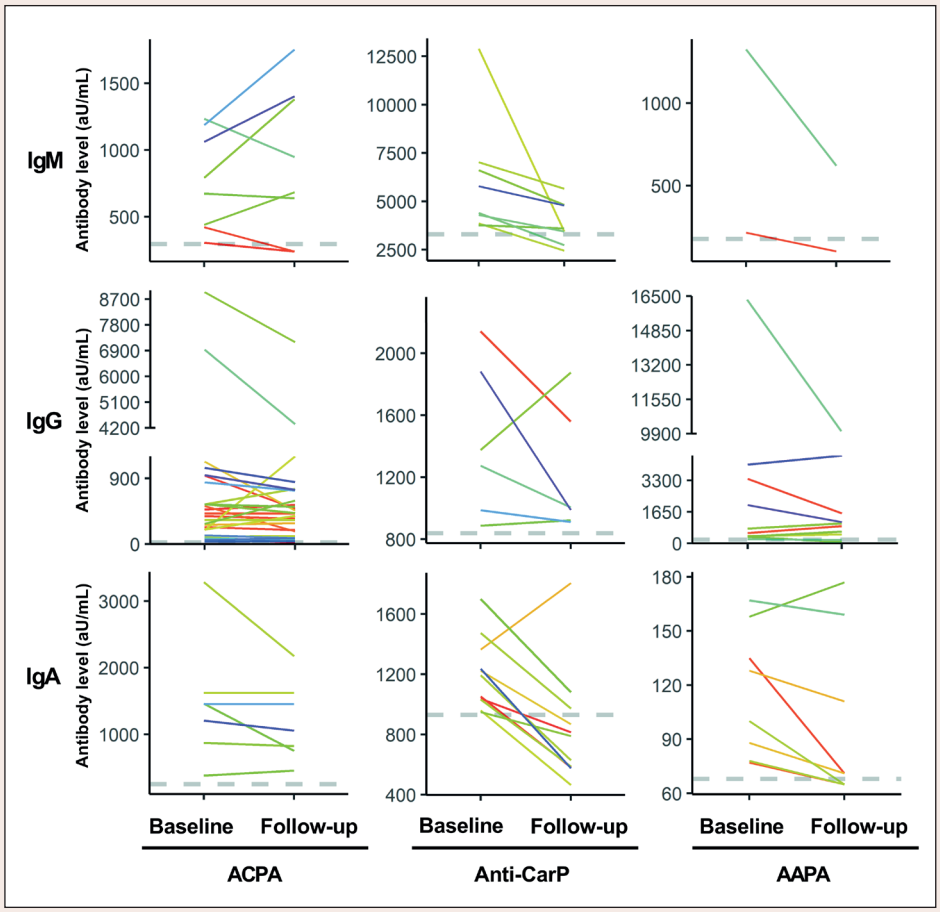
*RA defined as fulfilment of the 1987 and/or 2010 criteria at the time of clinically apparent inflammatory arthritis development. Of 55 patients with IA 42 (76%) fulfilled criteria for RA. Two patients of the non-RA group developed other diagnoses (1 inflammatory osteoarthritis, 1 psoriatic arthritis), and the remaining 11 patients had UA/clinical diagnosis of possible RA (though they did not fulfil classification criteria); nine patients received DMARD-therapy.
Figure A autoantibody negativity at baseline was defined as negative for the nine studied measurements (n=100), Figure B autoantibody positive was defined as at least one (out of nine) positive measurement at baseline (n=47).
Error bars in Figure A and B represent 95% CI. Dashed grey horizontal lines in Figure C indicate the cut-off values for each autoantibody.
No significant differences in Figure A and B were found. In patients with ≥1 autoantibody-reactivity at baseline progressing to RA, the median number of autoantibody-reactivities was 2.0 (IQR 1.0-4.0, max. 6) at baseline and 2.0 (IQR 1.0-4.0, max. 6) at RA-development (p=0.77). In CSA-patients with ≥1 autoantibody-reactivity at baseline not progressing to RA, this was 1.0 (IQR 1.0-2.0, max. 4) at baseline and 1.0 (IQR 0.0-2.0, max. 5) after 2-years (p=0.015). Levels of autoantibodies did not significantly change over time (p-values ranging 0.19-1.00).
ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies

Supplementary Figure 2. Changes in autoantibody-response over time: A) percentage of patients that has an increasing, decreasing or stable number of positive measurements over time in all CSA-patients (n=147), B) autoantibody levels over time in all CSA-patients.



Error bars in Figure A represent 95% CI. Dashed grey horizontal lines in Figure B indicate the cut-off values for each autoantibody.
Note that patients with measurements below detection range at both baseline and follow-up were illustrated as a horizontal line at the lower detection limit; consequently this depicted single line actually presents data of multiple patients.
No significant differences were found in Figure A between patients progressing and not progressing to IA. In patients with ≥1 autoantibody-reactivity at baseline progressing to IA, the median number of autoantibody-reactivities was 0.0 (IQR 0.0-1.0, max. 6) at baseline and 0.0 (IQR 0.0-1.0, max. 6) at IA-development (p=0.69). In CSA-patients with ≥1 autoantibody-reactivity at baseline not progressing to IA, this was 0.0 (IQR 0.0-0.0, max. 4) at baseline and 0.0 (IQR 0.0-0.0, max. 5) after 2-years (p=0.12). A significant decrease in autoantibody levels was seen in patients progressing from CSA to IA for ACPA IgA (p<0.001) and anti-CarP IgA (p=0.036). No significant changes in levels over time were seen in the remaining autoantibodies (p-values ranging 0.18-1.00).
IA: clinically apparent inflammatory arthritis, ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies

Supplementary Figure 3. Autoantibody levels over time in patients positive for the respective autoantibody at baseline, each colour indicates an individual patient.



Dashed grey horizontal lines indicate the cut-off values for each autoantibody.
ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies

Supplementary Table 4. Autoantibody levels in patients positive for ≥1 autoantibody-reactivity at baseline.

	ACPA IgM		ACPA IgG		ACPA IgA		CarP IgM		CarP IgG		CarP IgA		AAPA IgM		AAPA IgG		AAPA IgA	
IA	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**
-	<240	<240	<15	<15	NS	NS	<2400	<2400	246	199	***1038***	815	77	77	128	63	<65	<65
-	NS	NS	***471***	***536***	NS	NS	<2400	2835	<170	257	621	493	113	132	116	124	<65	<65
-	<240	<240	<15	<15	<120	<120	<2400	<2400	<170	<170	***1053***	583	61	64	<60	103	<65	<65
-	255	280	***522***	***176***	NS	NS	<2400	<2400	444	***1150***	729	***1151***	105	97	NS	142	***77***	<65
-	<240	<240	***43***	***56***	145	174	<2400	<2400	354	302	254	306	<60	<60	122	133	<65	<65
-	NS	***477***	***265***	***289***	NS	NS	<2400	<2400	599	494	292	429	70	71	140	165	<65	<65
-	<240	<240	<15	<15	NS	NS	2846	***4280***	340	606	***1364***	***1806***	74	101	82	109	***128***	***111***
-	<240	<240	<15	<15	NS	154	<2400	<2400	<170	239	660	611	66	79	NS	147	***88***	***71***
-	NS	<240	<15	<15	205	NS	***12892***	***3448***	249	308	***960***	466	NS	111	75	99	<65	<65
-	<240	<240	***327***	***332***	***1625***	***1621***	***3864***	2459	NS	420	618	792	90	68	NS	167	66	<65
-	NS	NS	***544***	***757***	***3287***	***2172***	***7025***	***5658***	715	391	***1193***	630	167	121	NS	NS	NS	NS
-	<240	<240	24	<15	NS	227	<2400	<2400	171	227	***1473***	***973***	67	<60	185	161	***78***	<65
-	<240	<240	<15	<15	191	156	<2400	<2400	539	361	347	364	92	75	NS	171	***100***	<65
-	<240	<240	<15	<15	NS	NS	<2400	<2400	294	217	339	448	<60	<60	166	147	***103***	NS
-	<240	<240	<15	<15	189	195	<2400	<2400	624	397	***949***	790	<60	<60	90	<60	<65	<65
-	<240	<240	<15	<15	136	<120	<2400	<2400	394	460	386	275	<60	<60	83	<60	***158***	***177***
-	<240	NS	***278***	***594***	***380***	***453***	<2400	3225	691	686	389	572	128	131	***345***	<60	<65	***76***
-	<240	<240	<15	<15	<120	<120	***3767***	***3605***	201	172	419	713	138	100	121	NS	<65	<65
-	<240	<240	<15	<15	155	154	***4399***	2732	187	173	474	392	138	139	<60	87	<65	<65

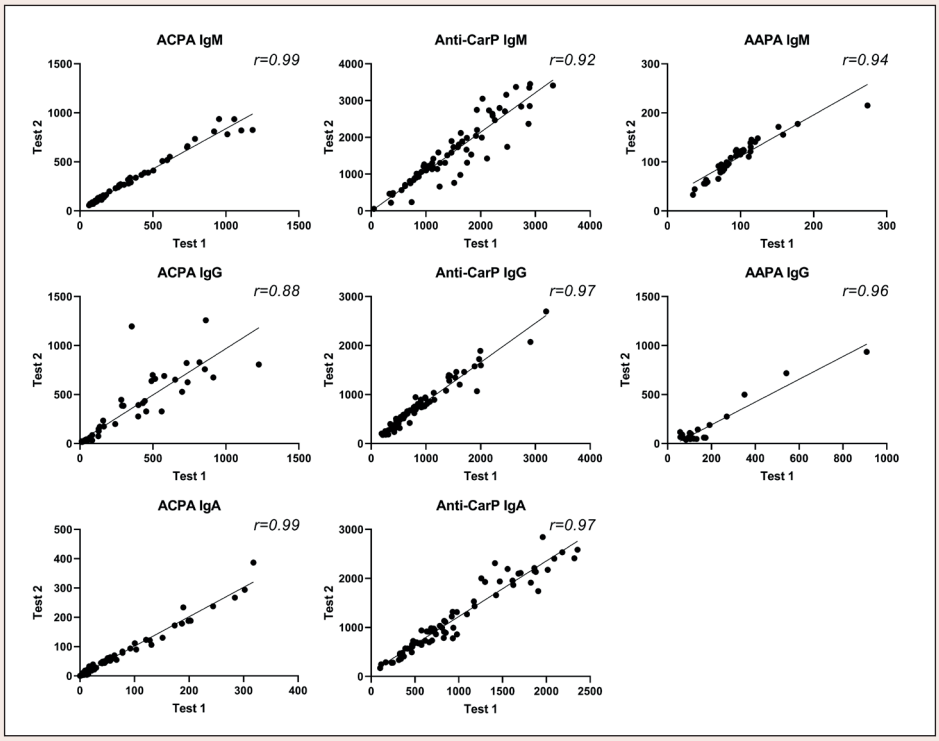
Supplementary Table 4. *Continued*

	ACPA IgM		ACPA IgG		ACPA IgA		CarP IgM		CarP IgG		CarP IgA		AAPA IgM		AAPA IgG		AAPA IgA	
IA	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**	**BL**	**FU**
-	NS	285	<15	<15	140	132	***4300***	***3448***	<170	<170	373	266	NS	138	68	<60	<65	<65
-	<240	<240	***83***	***43***	<120	<120	2529	2587	285	447	<250	<250	115	103	<60	<60	<65	<65
-	***1188***	***1754***	***845***	***733***	***1455***	***1454***	3022	<2400	***987***	***912***	491	752	<60	65	143	***381***	<65	<65
+	<240	<240	***81***	20	243	<120	<2400	<2400	593	504	505	473	69	66	72	84	<65	<65
+	***304***	<240	***939***	***487***	NS	NS	<2400	<2400	625	590	311	324	77	73	137	NS	<65	<65
+	<240	<240	***233***	***191***	130	<120	<2400	<2400	406	609	<250	<250	97	116	103	120	<65	<65
+	***421***	<240	***385***	***350***	NS	NS	3081	<2400	***2142***	***1560***	833	565	***215***	102	***3383***	***1577***	***135***	***71***
+	<240	<240	***419***	***422***	170	134	<2400	2520	646	498	<250	258	86	81	***542***	***909***	<65	<65
+	<240	<240	***1128***	***458***	NS	NS	<2400	<2400	623	541	***1223***	868	105	96	NS	NS	<65	<65
+	<240	***443***	***191***	***1200***	NS	***1465***	<2400	3263	413	***1212***	730	615	89	91	112	NS	<65	<65
+	<240	<240	***199***	***387***	NS	NS	<2400	<2400	336	346	397	405	100	105	118	98	<65	<65
+	<240	<240	***104***	***111***	NS	228	<2400	<2400	561	599	396	318	88	69	***377***	***475***	<65	<65
+	<240	<240	20	<15	166	<120	2837	***5185***	477	***1039***	578	<250	111	120	***212***	NS	<65	<65
+	<240	<240	<15	<15	251	235	<2400	<2400	274	763	***1032***	588	<60	64	NS	NS	<65	<65
+	***791***	***1380***	***8940***	***7194***	***870***	***822***	<2400	2834	***1376***	***1876***	374	599	82	83	***774***	***1053***	<65	<65
+	256	<240	***79***	***40***	192	183	***6613***	***4821***	NS	786	567	490	97	74	NS	198	<65	<65
+	***440***	***684***	***549***	***424***	NS	NS	2574	***3669***	502	616	922	776	86	92	<60	***621***	<65	<65
+	<240	<240	<15	<15	<120	<120	<2400	<2400	<170	<170	***1699***	***1080***	150	132	88	<60	<65	<65
+	***673***	***638***	***546***	***510***	***1459***	***750***	<2400	<2400	***887***	***923***	780	553	74	67	156	178	<65	<65

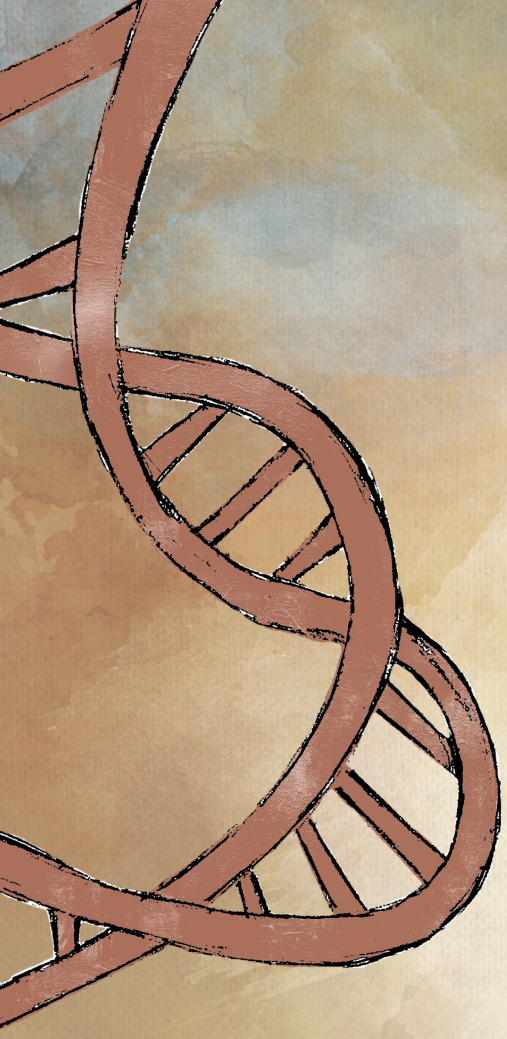
+	<240	<240	<15	<15	<120	<120	<2400	<2400	237	255	<250	<250	67	64	***218***	168	<65	<65
+	***1235***	***947***	***6931***	***4327***	NS	NS	2993	<2400	***1274***	***1005***	693	679	***1327***	***621***	***16338***	***10023***	***167***	***159***
+	<240	<240	***63***	***58***	<120	<120	<2400	<2400	509	535	<250	<250	105	87	100	154	<65	<65
+	<240	<240	***120***	***82***	247	125	<2400	<2400	253	216	279	277	110	113	76	128	<65	<65
+	<240	<240	***59***	***78***	138	125	<2400	***3389***	285	297	584	604	75	82	183	156	<65	<65
+	<240	<240	***34***	***42***	<120	<120	<2400	<2400	489	826	531	507	86	104	163	NS	<65	<65
+	<240	<240	<15	<15	NS	231	<2400	<2400	<170	<170	***1236***	577	<60	65	80	83	<65	<65
+	***858***	NS	***1045***	***850***	<1204	***1055***	***5779***	***4790***	446	288	547	540	134	NS	***4132***	***4596***	<65	NS
+	***1061***	***1403***	***946***	***743***	NS	NS	2976	2414	***1882***	***989***	689	NS	NS	NS	***2011***	***1120***	<65	<65

Switches within autoantibodies were defined as a decrease in IgM levels (level at follow-up < level at baseline) accompanied by an increase in IgG levels (level at follow-up > level at baseline) within the same autoantibody. Note that even small changes in autoantibody levels, including level changes below the cut-off, could result in a 'switch'. Switches were evaluated per autoantibody. In patients progressing to IA no switches occurred in ACPA and anti-CarP, and 5 patients (20%) switched in AAPA. In patients not progressing to IA no switches occurred in ACPA and AAPA, and 1 patient (4.5%) switched in anti-CarP.
IA: clinically apparent inflammatory arthritis, ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies, NS: non-specific measurement

Supplementary Figure 4. Inter-assay variation of in-house ELISAs



Inter-assay variation resulted in changes in positivity of the test infrequently: ACPA IgM 0%, IgG 1.3%, IgA 1.3%, anti-CarP IgM 9.2%, IgG 3.9%, IgA 7.9%, AAPA IgM 0%, IgG 4.2%, IgA 0%.
No correlation plot was created for AAPA IgA because too little samples were above the detection limit.
ACPA: anti-citrullinated protein antibodies, anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies



7

Determining in which pre-arthritis stage HLA-shared epitope alleles and smoking exert their effect on the development of rheumatoid arthritis

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Abstract

Objectives

The HLA-shared epitope-alleles (HLA-SE) and smoking are the most prominent genetic and environmental risk factors for rheumatoid arthritis (RA). However, at which pre-arthritis stage (asymptomatic/symptomatic) they exert their effect is unknown. We aimed to determine whether HLA-SE and smoking are involved in the onset of autoantibody-positivity, symptoms (Clinically Suspect Arthralgia, CSA) and/or progression to clinical arthritis.

Methods

We performed meta-analyses on results from literature on associations of HLA-SE and smoking with anti-citrullinated protein antibodies (ACPA) in the asymptomatic population. Next, we studied associations of HLA-SE and smoking with autoantibody-positivity at CSA-onset, and with progression to clinical inflammatory arthritis (IA) during follow-up. Associations in ACPA-positive CSA-patients were validated in meta-analyses with other arthralgia-cohorts. Analyses were repeated for rheumatoid factor (RF), anti-carbamylated and anti-acetylated antibodies (anti-CarP, AAPA).

Results

Meta-analyses showed that HLA-SE is not associated with ACPA-positivity in the asymptomatic population (OR 1.06 (95%CI;0.69-1.64)), whereas smoking was associated (OR 1.37 (1.15-1.63)). At CSA-onset, both HLA-SE and smoking associated with ACPA-positivity (OR 2.08 (1.24-3.49), OR 2.41 (1.31-4.43)). During follow-up, HLA-SE associated with IA-development (HR 1.86 (1.23-2.82)), in contrast to smoking. This was confirmed in meta-analyses in ACPA-positive arthralgia (HR 1.52 (1.08-2.15)). HLA-SE and smoking were not associated with RF, anti-CarP or AAPA-positivity at CSA-onset. Longitudinally, AAPA associated with IA-development independent from ACPA and RF (HR 1.79 (1.02-3.16)), anti-CarP did not.

Conclusions

HLA-SE and smoking act at different stages: smoking confers risk for ACPA- and symptom-development, whereas HLA-SE mediates symptom- and IA-development. These data enhance the understanding of the timing of the key risk factors in development of RA.

Introduction

The HLA shared epitope (SE) is the most well-known and strongest genetic risk factor for development of rheumatoid arthritis (RA), especially for anti-citrullinated protein antibody (ACPA)-positive RA.[1-14] Similarly, smoking is the strongest environmental risk factor for autoantibody-positive RA[2,9,10,12,15]; multiple studies have shown this effect is mostly present in people carrying HLA-SE alleles.[1,3,5,6,8,14,16] This knowledge is mostly obtained from case-control studies comparing RA-patients and healthy controls. During the last decade, research attention has shifted to the stages that precede clinical arthritis and RA and several pre-RA stages have been discerned. However, so far it remains undetermined at which stage(s) HLA-SE alleles and smoking exert their effect.

The following stages are distinguished. An asymptomatic stage in which autoimmune responses can develop, resulting in autoantibody-positivity. Then, autoimmune responses can mature and a symptomatic stage develops. The pattern of symptoms that is considered specific for an increased risk of RA is called clinically suspect arthralgia (CSA). Patients with CSA can progress to clinically apparent inflammatory arthritis (IA); the stage when RA is generally diagnosed.[17] This model suggests that genetic factors exert their influence first, followed by smoking with subsequent autoantibody-development.[17,18] However, this time-order has never been shown.

In addition to a nested case-control study,[19] several longitudinal studies assessed genetic factors and/or smoking and provided data either from healthy to IA but not the intermediate stages, or from mixed populations of asymptomatic and symptomatic people.[20-24] These approaches do not allow determination of stage-dependent effects. As for the asymptomatic stage, contrasting findings are reported on associations between HLA-SE alleles and smoking and the presence of ACPA in the general population.[2,14,25-28] To the best of our knowledge only one study evaluated the effect of smoking on the progression from ACPA-positivity to CSA.[29] Furthermore, longitudinal studies within arthralgia are scarce and their findings varied.[30,31] The mentioned studies focused on ACPA, however, HLA-SE and smoking might also interact with other autoantibodies such as rheumatoid factor (RF), anti-carbamylated (anti-CarP) and anti-acetylated (AAPA) protein antibodies, the time-effects of which have not yet been studied.

We aimed to determine at which pre-RA stage HLA-SE and smoking exert their effect by studying both original and previously reported data. More specifically, we performed meta-analyses on literature from the general population, analyzed our own data at CSA-onset and during progression to IA, and finally performed meta-

analyses using data from different longitudinal arthralgia-cohorts. In doing this we focused on fine-staging the effects in the development of ACPA-positive RA. Analyses were repeated for ACPA-negative RA and associations of RF, anti-CarP and AAPA.

Methods

Summarizing literature obtained from the general population

The literature was reviewed on studies reporting associations between HLA-SE and/or smoking with the presence of ACPA in the asymptomatic population, as described supplementary. Results were pooled in meta-analyses. Although these studies were cross-sectional in nature, observed findings were considered to reflect the influence of HLA-SE/smoking on ACPA-development, as this is most likely the first event in the development of ACPA-positive RA.

The symptomatic phase

Associations of HLA-SE and smoking with autoantibodies at CSA-onset were investigated in the Leiden CSA-cohort, we did not identify large cohorts for validation since most arthralgia-cohorts did not include autoantibody-negative patients. Additionally, the role of HLA-SE and smoking in progression from arthralgia to IA was investigated in the Leiden CSA-cohort. Results obtained in the ACPA-positive subgroup were validated in ACPA-positive arthralgia/at-risk-patients from two independent cohorts (Amsterdam, Leeds).

Measurements at CSA-onset

Patients presenting with CSA to the Leiden rheumatology outpatient clinic between April 2012-September 2019 were studied. As described in detail previously,[32] patients had recent-onset (<1 year) arthralgia of small joints and were, according to the clinical expertise and pattern recognition of the rheumatologist, at risk for progression to RA. Patients were excluded if clinical arthritis was already present, or if a different explanation for the joint pain was more likely. At baseline smoking-status (present/past/never) was obtained through questionnaires. Presence of IgM RF (in-house ELISA, cut-off >3.5 IU/mL) and IgG ACPA (anti-CCP2, Phadia, Nieuwegein, the Netherlands, cut-off >7 IU/mL) was determined during routine laboratory measurements in all patients, presence of IgG anti-CarP and IgG AAPA with in-house ELISA in a subset of patients. Detailed methods are described supplementary. The HLA-SE alleles were extracted from whole genome sequencing data; the HLA-region was isolated and imputed using the SNP2HLA software and T1DGC reference panel.[33] HLA-SE positivity was subsequently defined as the presence of 1 or 2 of the HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408 and *1001 (see supplementary material).[34]

Measurements on the progression from CSA to IA

Patients in the Leiden CSA-cohort were prospectively followed (median (IQR) 106 weeks (43-114)) for development of IA, which was defined as ≥1 swollen joints at physical examination by a rheumatologist. Treatment with disease-modifying anti-rheumatic drugs (DMARDs, including systemic or intra-articular corticosteroids) was not allowed before IA-development. Analyses evaluating progression to IA were stratified for ACPA-status and results from the ACPA-positive subgroup were studied in meta-analyses with the results from ACPA-positive patients included in the Amsterdam and Leeds cohorts. The Amsterdam cohort included ACPA- and/or RF-positive patients; for this study the data from ACPA-positive arthralgia-patients was obtained and studied.[31] Data on smoking history, presence of HLA-SE, RF, ACPA and anti-CarP were collected previously and are described supplementary. In addition, IgG AAPA was determined in baseline serum samples simultaneous with Leiden CSA-samples. Results on predictive value of HLA-SE and smoking in ACPA-positive patients from the Leeds cohort were obtained from Rakieh et al.,[30] detailed methods are described supplementary. Anti-CarP and AAPA were not determined in the Leeds cohort.

In sub-analyses, the association of HLA-SE and smoking with RA-development was studied using Leiden CSA-data; RA was defined as development of IA plus fulfillment of the 1987 and/or 2010 EULAR/ACR criteria at that time.[35,36]

Statistics

Results from literature on associations of HLA-SE and smoking with ACPA in the asymptomatic population were pooled in inverse-variance weighted meta-analyses.

Associations of HLA-SE and smoking with autoantibody-positivity at CSA-onset were investigated with logistic regression analyses. Results of smoking were also stratified for HLA-SE. Associations of HLA-SE and smoking with ACPA-level in ACPA-positive patients were evaluated with Mann-Whitney U tests and logistic regression.

Associations with IA-development were studied with cox regression, also stratified for ACPA. Results in ACPA-positive arthralgia were summarized in inverse-variance weighted meta-analyses.

Associations of anti-CarP and AAPA with IA-development were corrected for concomitant ACPA- and RF-positivity in multivariable analyses with the autoantibody-negative group as reference in the Leiden data (the Amsterdam cohort did not include autoantibody-negative patients). The additional value of anti-CarP and AAPA to ACPA- and RF-positivity for prediction of IA-development was determined in the ACPA+RF+

subgroup from the Leiden and Amsterdam cohorts.

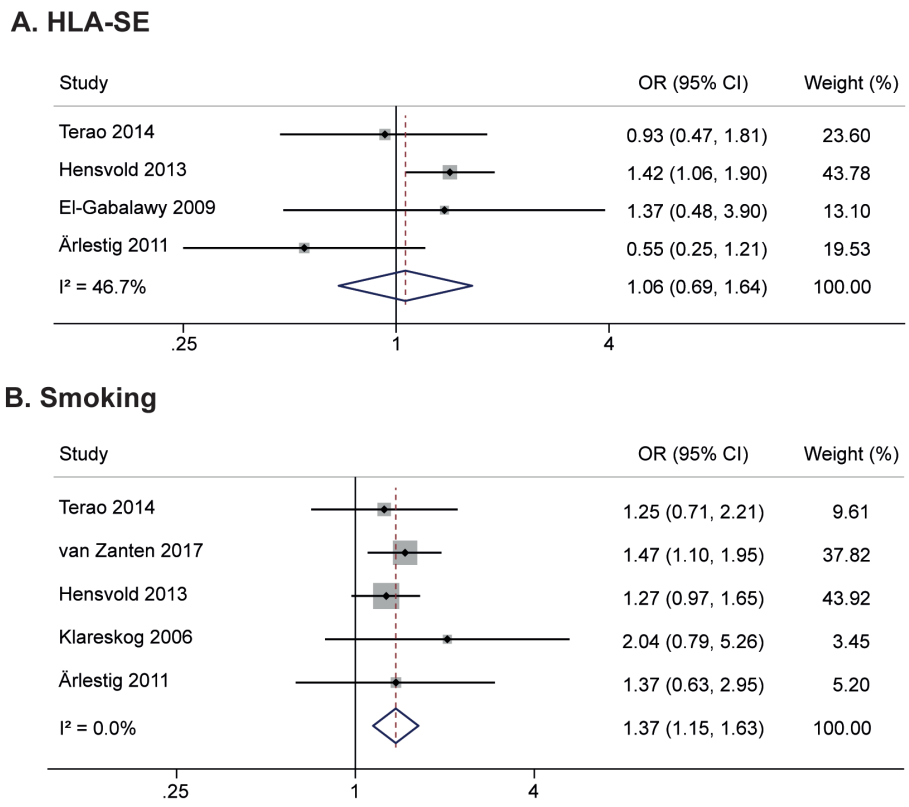
P-values <0.05 were considered statistically significant. IBM SPSS Statistics (V25) and STATA (V16) were used.

Results

Summarizing literature obtained from the asymptomatic stage

Four studies were identified on the association of HLA-SE with ACPA, and five on smoking (Supplementary File 1). Meta-analyses revealed that HLA-SE was not associated with ACPA-positivity (OR 1.06 (95%CI;0.69-1.64)), whereas smoking was associated (OR 1.37 (1.15-1.63)), Figure 1. This suggests that smoking, but not HLA-SE, conferred risk for ACPA-development in the asymptomatic stage.

Figure 1. Meta-analyses on HLA-SE (A) and smoking (B) in asymptomatic healthy individuals and first-degree relatives, showing associations with presence of ACPA for smoking but not for HLA-SE



HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, OR: odds ratio, CI: confidence interval

Associations with ACPA at CSA-onset

Characteristics of patients presenting with CSA (n=577) are provided in the supplementary materials. HLA-SE positive CSA-patients were more often ACPA-positive (OR 2.08 (95%CI;1.24-3.49), this relation was dependent on the number of alleles (Table 1). Patients that smoked were also more often ACPA-positive (OR 2.41 (1.31-4.43)), which was also dose-dependent with an higher OR for current smokers than ex-smokers (Table 1). In addition, within smokers, it was dependent on number of packyears, because the odds for being ACPA-positive increased per increase in packyear (OR 1.03 (1.00-1.06)). As it has been reported in RA that the association of smoking is dependent on HLA-SE status, we stratified the analyses of smoking (ever versus never) for HLA-SE; smoking was associated with ACPA-status in both HLA-SE negative and HLA-SE positive CSA-patients (Table 1). The association of HLA-SE and smoking with ACPA-positivity was present for both ACPA double-positivity (ACPA+RF+) and single-positivity (ACPA+RF-), and thus independent from RF (Supplementary Table 2). Studying the levels of ACPA within ACPA-positive patients at CSA-onset revealed that HLA-SE positive patients tended to have higher levels than HLA-SE negative patients (median (IQR) 236 (72-340) versus 144 (32-340), p=0.12), whilst no effect on ACPA-levels was present for smoking (229 (64-340) versus 222 (52-340), p=0.89), see Supplementary Table 3 for results from regression analyses.

Table 1. Associations of HLA-SE and smoking with presence of ACPA in patients newly presenting with CSA

		ACPA positive, n (%)	ACPA negative, n (%)	OR (95% CI)	p-value
All patients					
HLA-SE	Absent	27 (39)	259 (57)	Reference	--
	Present	42 (61)	194 (43)	2.08 (1.24-3.49)	0.006
HLA-SE	0	27 (39)	259 (57)	Reference	--
	1	31 (45)	161 (36)	1.85 (1.06-3.21)	0.029
	2	11 (16)	33 (7)	3.20 (1.45-7.04)	0.004
Smoking	Never	15 (23)	185 (42)	Reference	--
	Ever	49 (77)	251 (58)	2.41 (1.31-4.43)	0.005
Smoking	Never	15 (23)	185 (42)	Reference	--
	Ex-smoker	28 (44)	161 (37)	2.15 (1.12-4.16)	0.024
	Current smoker	21 (33)	90 (21)	2.88 (1.42-5.85)	0.003

Table 1. *Continued*

		ACPA positive, n (%)	ACPA negative, n (%)	OR (95% CI)	p-value
HLA-SE positive subgroup					
Smoking	Never	10 (27)	77 (45)	Reference	--
	Ever	27 (73)	95 (55)	2.19 (1.00-4.80)	0.051
Smoking	Never	10 (27)	77 (45)	Reference	--
	Ex-smoker	13 (35)	57 (33)	1.76 (0.72-4.29)	0.22
	Current smoker	14 (38)	38 (22)	2.84 (1.15-6.98)	0.023
HLA-SE negative subgroup					
Smoking	Never	4 (18)	99 (43)	Reference	--
	Ever	18 (82)	130 (57)	3.43 (1.12-10.45)	0.030
Smoking	Never	4 (18)	99 (43)	Reference	--
	Ex-smoker	11 (50)	89 (39)	3.06 (0.94-9.95)	0.063
	Current smoker	7 (32)	41 (18)	4.23 (1.17-15.22)	0.027

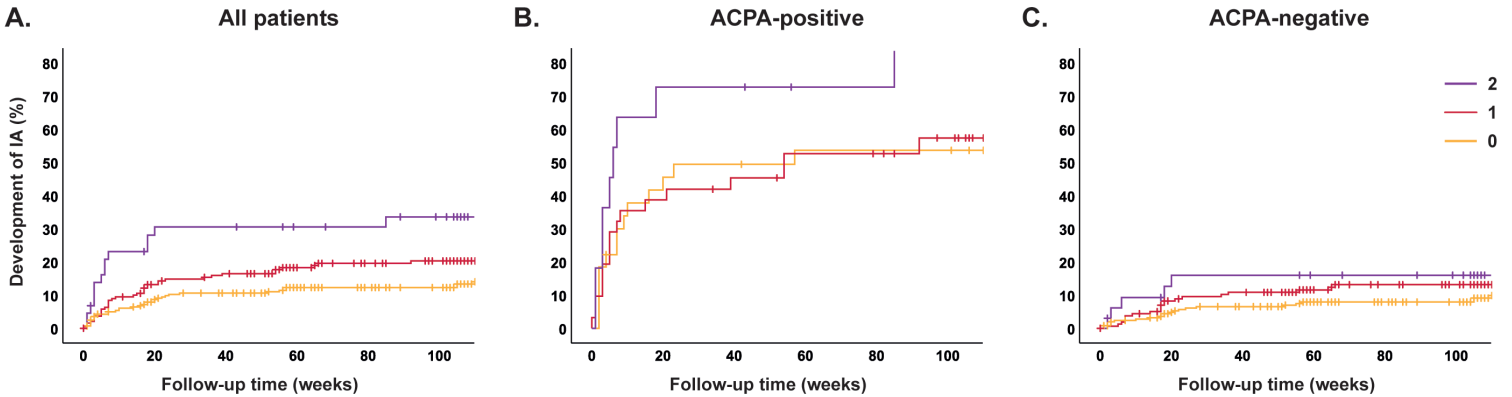
*Numbers on smoking in HLA-SE strata do not add up to numbers in the total CSA-group as some patients with data on smoking have missing data on HLA-SE.
HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, CSA: clinically suspect arthralgia, OR: odds ratio, CI: confidence interval

Progression to IA in ACPA-positive CSA

Patients were followed for development of IA; median time till IA was 16 weeks (IQR 3-36), non-progressors were followed for median 109 (62-116) weeks. Presence of HLA-SE was significantly associated with IA-development in all CSA-patients (HR 1.86 (95%CI;1.23-2.82)), also here a dose-response relation was present (Figure 2A, Supplementary Table 4). Within the ACPA-positive subgroup the HR was 1.29 (0.67-2.47, Figure 2B, Supplementary Table 4). Because of the small sample size after stratification and risk of type-II error, we performed meta-analysis including ACPA-positive patients from two other arthralgia-cohorts. This showed that HLA-SE significantly associated with IA-development in ACPA-positive patients (HR 1.52 (1.08-2.15), Figure 4A).

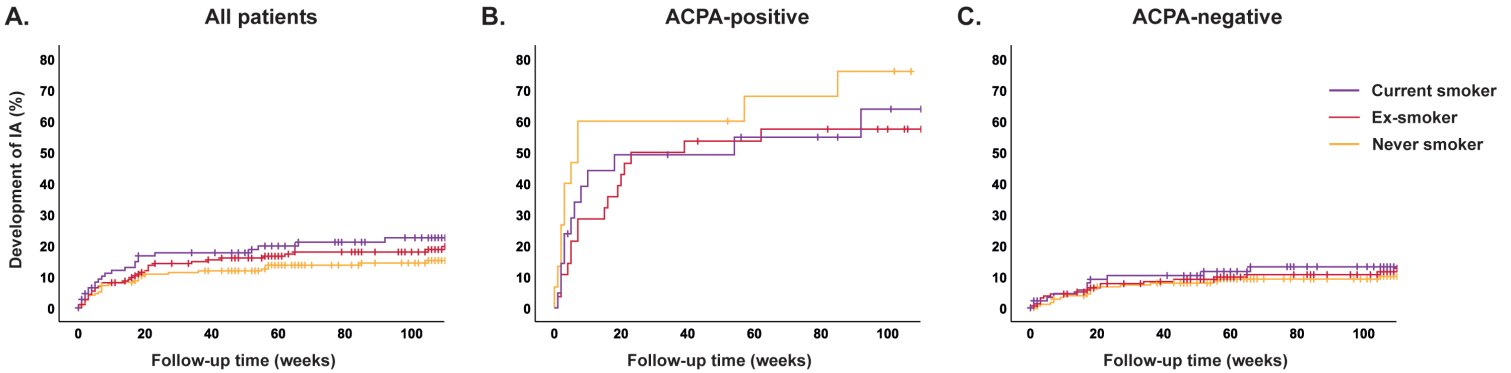
Smoking was not associated with IA-development, neither in the total CSA-population (HR 1.40 (0.90-2.18), Figure 3A, Supplementary Table 5), nor in the ACPA-positive subgroup (HR 0.59 (0.29-1.18), Figure 3B, Supplementary Table 5), nor in meta-analysis including ACPA-positive patients from three cohorts (HR 0.94 (0.67-1.33), Figure 4B).

Figure 2. Associations of number of HLA-SE alleles (0/1/2 alleles present) with progression from CSA to inflammatory arthritis



Corresponding hazard ratios, with 0 HLA-SE alleles as reference category were: (A) HR 1.65 (95% CI 1.06-2.56) and HR 3.03 (1.64-5.61) for 1 and 2 HLA-SE alleles respectively, (B) HR 1.05 (0.52-2.13) and HR 2.32 (1.00-5.41), and (C) HR 1.66 (0.94-2.94) and HR 2.00 (0.76-5.28), see Supplementary Table 4.
HLA-SE: shared epitope, CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, IA: inflammatory arthritis, HR: hazard ratio, CI: confidence interval

Figure 3. Associations of smoking with progression from CSA to inflammatory arthritis



Corresponding hazard ratios, with never smoker as reference category were: (A) HR 1.25 (95% CI 0.76-2.06) and HR 1.66 (0.97-2.83) for ex-smoker and current smoker respectively, (B) HR 0.55 (0.26-1.19) and HR 0.64 (0.28-1.45), and (C) HR 1.17 (0.61-2.24) and HR 1.56 (0.76-3.18), see Supplementary Table 5.
CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, IA: inflammatory arthritis, HR: hazard ratio, CI: confidence interval

Thus HLA-SE, but not smoking, influenced the risk to progress from ACPA-positive CSA to RA.

Associations of HLA-SE and smoking in ACPA-negative CSA

Presence of HLA-SE was associated with IA-development in ACPA-negative patients (HR 1.71 (0.99-2.96)), although the CI just included 1 (Figure 2C, Supplementary Table 4). Within ACPA-/RF- and ACPA-/RF+ CSA-patients associations of HLA-SE with IA-development were HR 1.64 (0.90-2.99) and HR 2.07 (0.55-7.75), respectively.

The tendency of HLA-SE to associate with IA-development in ACPA-negative patients disappeared in sensitivity analyses with the outcome RA, in contrast to the effect that remained within ACPA-positive patients (Supplementary Figure 3). Hence, HLA-SE was not convincingly associated with progression from symptoms to IA in ACPA-negative patients.

Smoking did also not associate with progression to IA in ACPA-negative patients (HR 1.30 (0.73-2.33)), Figure 3C, Supplementary Table 5.

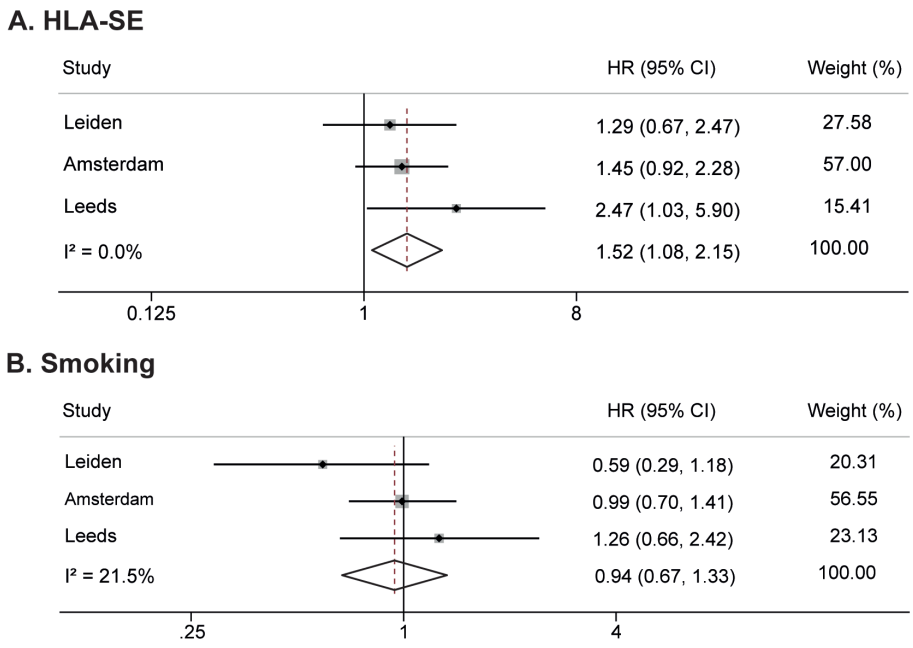
Associations of HLA-SE and smoking with anti-CarP and AAPA at CSA-onset

Neither HLA-SE positivity nor smoking was associated with a higher frequency of RF, anti-CarP or AAPA at presentation with CSA, both in univariable analyses and after correction for concomitant presence of ACPA (Supplementary Table 6).

Associations of anti-CarP and AAPA with IA-development

In univariable analyses, anti-CarP and AAPA were associated with IA-development (Table 2). Correcting for ACPA and RF in the Leiden cohort revealed that AAPA was significantly associated with RA-development, but anti-CarP was not. Similar multivariable analyses were not possible in the Amsterdam cohort because of the lack of an autoantibody-negative reference group. Instead, we studied the association of both AMPA's in the ACPA+/RF+ subgroups. Meta-analyses of data from the two cohorts revealed a significant association for AAPA (HR 1.53 (1.02-2.28)), but not for anti-CarP (HR 1.29 (0.85-1.97), Figure 5).

Figure 4. Meta-analyses on HLA-SE (A) and smoking (B) in three cohorts of ACPA-positive arthralgia patients, showing an association with clinical arthritis development for HLA-SE but not for smoking



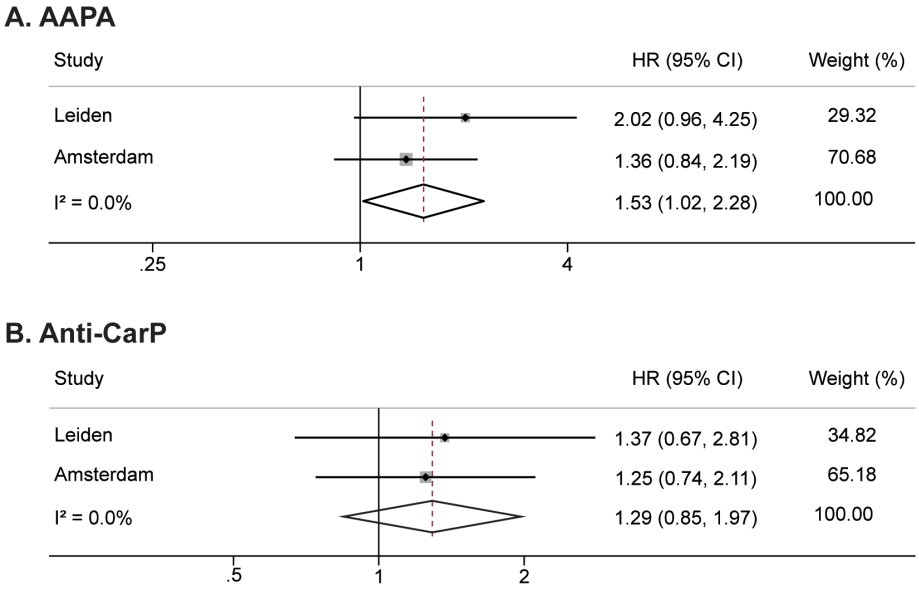
Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt et al. were obtained and analysed. Results from the Leeds cohort were obtained from Rakieh et al. (Table 2 from reference[30]).
HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, HR: hazard ratio, CI: confidence interval, CSA: clinically suspect arthralgia

Table 2. Associations of autoantibodies with development of inflammatory arthritis in patients newly presenting with arthralgia

	Univariable cox regression		**Multivariable cox regression**		**Multivariable cox regression**	
CSA cohort	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*	*HR (95% CI)*	*P-value*
ACPA IgG	3.29 (2.11-5.13)	<0.001	2.55 (1.44-4.53)	0.001	2.97 (1.73-5.10)	<0.001
RF IgM	1.72 (1.11-2.67)	0.015	1.01 (0.61-1.69)	0.96	0.98 (0.58-1.67)	0.95
AAPA IgG	3.07 (1.90-4.98)	<0.001	1.79 (1.02-3.16)	0.043	--	--
Anti-CarP IgG	2.85 (1.59-5.11)	<0.001	--	--	1.47 (0.75-2.87)	0.26

ACPA: anti-citrullinated protein antibody, RF: rheumatoid factor, AAPA: anti-acetylated protein antibody, anti-CarP: anti-carbamylated protein antibody, HR: hazard ratio, CI: confidence interval

Figure 5. Meta-analyses on AAPA (A) and anti-Carp (B) in two cohorts of ACPA-positive/RF-positive arthralgia patients, showing an association with IA-development for AAPA but not for anti-CarP

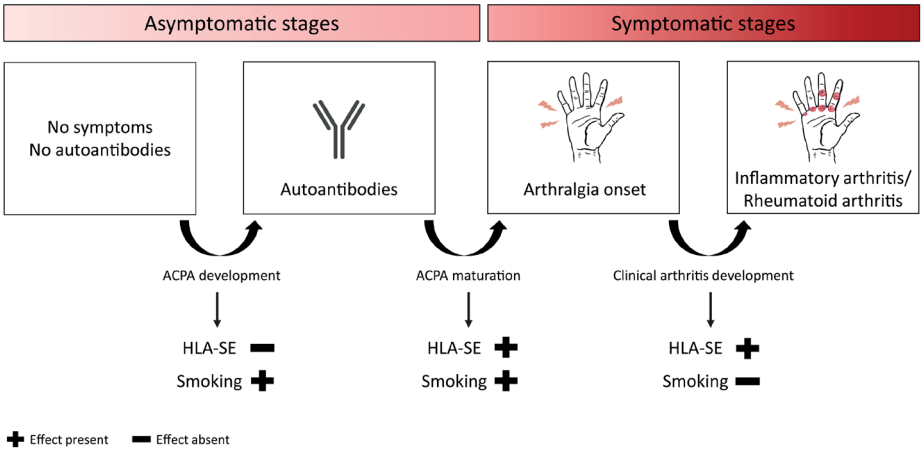


Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt et al. were obtained and analysed.
AAPA: anti-acetylated protein antibody, anti-CarP: anti-carbamylated protein antibody, RF: rheumatoid factor, ACPA: anti-citrullinated protein antibody, HR: hazard ratio, CI: confidence interval, CSA: clinically suspect arthralgia

Discussion

Although it has been extensively shown that HLA-SE and smoking are risk factors for RA, it was thus far unclear in which pre-arthritis stage these factors exert their effect. We aimed to fine-stage the effects of HLA-SE and smoking, taking advantage of our own cohort data, as well as published data. Results from meta-analyses in people in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in development of ACPA. At CSA onset, both HLA-SE and smoking were associated with the presence of ACPA, although only HLA-SE associated with progression towards arthritis and RA. Presuming that autoantibody-development as a proxy for the emerging autoimmune response, is the first event, these results imply that smoking is involved in autoantibody-development and possibly symptom-development, but not with further IA-development. In contrast, HLA-SE is not involved in initial autoantibody-development, but rather associated with autoantibody-maturation and symptom-development as implied by results found at CSA-onset. Furthermore, it associates with further progression to clinical disease (Figure 6).

Figure 6. Summary of results on the role of HLA-SE and smoking in the asymptomatic and symptomatic phase of rheumatoid arthritis development



Meta-analyses in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in development of ACPA. At CSA onset, both HLA-SE and smoking were associated with presence of ACPA. Only HLA-SE further stimulated progression towards arthritis and ACPA-positive RA. Together these data imply that smoking is involved in autoantibody- and symptom-development, HLA-SE plays a role in autoantibody-maturation, symptom-development and progression to clinical disease.

To evaluate the role of HLA-SE and smoking in the asymptomatic phase we reviewed the literature following PRISMA guidelines for systematic literature reviews as much as possible (Supplementary File 1).[37] The results of identified studies performed in asymptomatic populations were combined in meta-analyses. These revealed an effect for smoking and absence of an association of HLA-SE with ACPA-positivity. Recent data in RA-patients indicated that smoking does not associate with ACPA as such, but rather with RF or autoantibodies in general.[6,15,16,38,39] Although not all of the studies included in the meta-analyses contained data on RF, pooled analysis did not identify an association between smoking and RF in the asymptomatic population (supplementary material). Also in CSA-patients no association between RF and smoking was found. All included studies were cross-sectionally performed in the general population. As we presumed that ACPA-positivity is the first event in the development of ACPA-positive RA, we believe the observed findings reflect effects of HLA-SE and smoking on autoantibody-development.

For smoking an association with ACPA was found at the asymptomatic stage and at CSA-onset. Our analyses at CSA-onset were cross-sectional in nature; therefore we cannot definitely conclude whether smoking truly associates with progression from autoantibody-positivity to symptom-development (alternatively, the association found at CSA-onset could be reflective of the association with ACPA-development). However, one longitudinal study evaluated ACPA-positive individuals from the

general population until development of CSA and showed a significant association of smoking with CSA-development.[29] Together with our data this suggests that smoking plays a role in the development of ACPA, further maturation and symptom-development.

The absence of an association of HLA-SE with ACPA in the asymptomatic population, the presence of this association at CSA-onset, and the finding that ACPA-levels tended to be higher in HLA-SE positive CSA-patients (which is in line with a previous study on ACPA-levels in arthralgia[40]), suggest that HLA-SE associates with maturation of the ACPA-response and/or symptom-onset. However, the latter implication is based on deductions from cross-sectional data, longitudinal data from ACPA-positivity to symptom-onset would have been preferable.

Several nested case-control studies have shown that autoantibody-development and the increase in levels can occur years before disease-onset.[41-43] The current study and previous studies on CSA showed that the period between CSA-onset and clinical arthritis development is on average 4-6 months.[44] We recently showed that the autoantibody-response had already matured at CSA-onset and did not mature further towards RA-development.[45] Together these results indicate that autoantibody-response maturation took place before symptom-onset, and was influenced by smoking and HLA-SE. However, although case-control studies have found gene-environment interactions,[6,9,10,14] we found no statistically signification interaction between HLA-SE and smoking for presence of ACPA at CSA-onset (p=0.52). Interestingly, in the asymptomatic phase ACPA-positivity can serorevert to negativity, as is shown in symptom-free relatives of RA-patients.[23] This is in contrast to what is described in the symptomatic phases of CSA and clinical RA,[45-48] where autoantibody-status and -levels were shown to be stable and seroreversion was infrequent. Regarding time-lines, this suggests that the autoimmune response is no longer reversible at symptom-onset. However, disease chronicity is then not yet established; only a proportion of CSA-patients develop RA, and both joint symptoms and subclinical inflammation can resolve spontaneously, also in ACPA-positive patients.[49] The final processes resulting in irreversible ACPA-positive RA remain to be elucidated. However the current data also suggest that this final step is influenced by HLA-SE.

This is not the first longitudinal study on HLA-and smoking and the progression from arthralgia to clinical arthritis. We took advantage of existing data to strengthen the findings and show consistency in the ACPA-positive group. Furthermore, the fact that the Leiden CSA-cohort included patients based on the clinical phenotype and not on autoantibody-status, ensured inclusion of also autoantibody-negative CSA-patients. This served to explore the role of HLA-SE and smoking in ACPA-negative RA.

Although, HLA-SE seemed to promote IA-development in ACPA-negative patients; this effect was not present for RA-development as outcome. Large case-control studies have suggested a role for HLA-SE also in ACPA-negative RA albeit with a smaller effect size than in ACPA-positive RA.[50] The present longitudinal data on ACPA-negative IA- or RA-development were insufficient to support a role for HLA-SE in the symptomatic pre-RA stage.

This study focused on associations of ACPA as measured with anti-CCP2, associations with other ACPA-tests (e.g. anti-CCP3) were not studied. However, in addition to ACPA, we did evaluate other AMPA's. Although different studies have shown cross-reactivity between ACPA and other AMPA's.[51,52] associations with HLA-SE and smoking at CSA-onset seemed to be specific for ACPA as no such associations were found for AAPA and anti-CarP in our patient population. This is in line with findings in RA, where anti-CarP was also not associated with HLA-SE and smoking.[53]

We aimed to fine-stage the effects of HLA-SE and smoking. Identification of predictive markers for IA- or RA-development in CSA was not our primary aim. Nonetheless, we included an exploration and observed that AAPA, but not anti-CarP, associated with IA, independent of ACPA and RF. Further research is needed to ascertain the diagnostic value of these autoantibodies, especially their relevance on top of ACPA and RF that are measured in daily practice.

This study has extended knowledge on the timing of HLA-SE and smoking in the different stages of RA-development. Intriguingly, HLA-SE and smoking exert their effect in partly different phases. Although requiring further biological exploration, it is tempting to speculate that initial autoantibody-development is stimulated by smoking, whereas further expansion of the autoimmune response is promoted differently; by an HLA-SE-restricted T-cell reaction, that drives further ACPA-response maturation. As such, smoking may contribute to development of autoantibodies in general.[6,15,16,38,39] This initial antibody-development does, most likely, require T-cell help as the antibodies are of the IgG isotype and hence the antibody producing B-cells have undergone isotype-switching, a T-cell dependent process. However, as no association with the HLA-system is observed at this stage, these T-cells most likely act in a HLA-SE-independent manner. In contrast, the subsequent expansion of the ACPA-response does associate with HLA-SE, indicating that another, second, T-cell response is involved in the further expansion of the ACPA-response. These T-cells are associated with HLA-SE and, conceivably, recognize other antigens than the ones involved in the T-cell response underlying the "initial" ACPA-response. Thereafter, ACPA-positive persons with HLA-SE are particularly prone for further progression towards RA. These insights in timing of environmental and genetic factors support a

further refinement of the SE-hypothesis; the HLA-SE specific T-cell response may not promote the initial break of tolerance to citrullinated-antigens, but rather promotes the expansion of the (already existing) ACPA-response prior to disease-onset. Conceptually, this would explain why ACPA-positive patients with HLA-SE develop RA more often than ACPA-positive patients without HLA-SE, and why HLA-SE does not associate with the other autoantibodies.

The findings of our study can guide future prevention studies. Prevention often concentrates on health-promoting behaviors. Our results on smoking imply that cessation of smoking might be able to influence the risk of ACPA-development and/or symptom-onset, but also that it may not be effective in reducing the risk of progression from CSA to clinical arthritis. This would mean that trials on smoking cessation might preferably assess the efficacy in disease prevention in the asymptomatic population (primary prevention), rather than in arthralgia-patients (secondary prevention).

To conclude, HLA-SE and smoking act in partly different pre-RA stages. Smoking confers risk for development of ACPA and/or joint symptoms, but does not further associate with IA-development. In contrast, HLA-SE does not associate with ACPA in the general population, but does mediate symptom-development and progression to IA. Even though the underlying time-specific biological pathways need further exploration, these data enhance understanding of timing of key genetic and environmental risk factors in development of RA.

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Supplementary File 1 – Literature and meta-analyses

A literature search was performed to investigate associations between HLA-SE, smoking and anti-citrullinated protein antibodies (ACPA) in the asymptomatic general population and first-degree relatives (FDR). See Supplementary Figure 1 for a flowchart of the article selection. PubMed was searched until September 2020 with terms as: "SE"[All Fields] AND ("smoke"[MeSH Terms] OR "smoke"[All Fields] OR "smoke s"[All Fields] OR "smoked"[All Fields] OR "smokes"[All Fields] OR "smoking"[MeSH Terms] OR "smoking"[All Fields] OR "smokings"[All Fields] OR "smoking s"[All Fields]) AND ("autoantibodies"[MeSH Terms] OR "autoantibodies"[All Fields] OR "autoantibody"[All Fields]) AND ("healthies"[All Fields] OR "healthy"[All Fields]), including different combinations of SE, shared epitope, smoking, autoantibodies, ACPA, healthy, asymptomatic, preclinical, general population, population-based and FDR (see below for all combinations used). Additional articles were identified by hand searching reference lists. After removal of reviews, duplicates and articles that did not apply to our research question (based on title and abstract screening), ten articles remained. Subsequently all studies that were either cross-sectional or longitudinal studies and contained information on associations between HLA-SE, smoking and ACPA-development were considered eligible. No longitudinal studies starting the healthy population were identified; all identified studies were cross-sectional in nature. However, since ACPA-development is most likely the first event in development of ACPA-positive disease, we believe the observed findings in cross-sectional studies reflect effects of HLA-SE and smoking on ACPA-development. The ten selected articles were further studied on the description of the population. Studies evaluating populations in which part of subjects had swollen and/or tender joints, and studies in which associations with autoantibodies were not investigated for ACPA (separate from other autoantibodies) were excluded. Finally, six cross-sectional studies were eligible for meta-analyses. Four studies evaluated associations between HLA-SE and ACPA,[1-4], and five studies evaluated associations between smoking and ACPA.[1,2,4-6] Meta-analyses were first performed without a stringent evaluation of study bias and thereafter with assessing this bias as recommended according to the PRISMA guidelines.[7] First, the odds ratios (OR) of these four and five studies respectively were combined in inverse-variance weighted meta-analyses, I^2 was determined to evaluate heterogeneity across the studies. Some studies were truly population based (no selection), these were the studies of van Zanten et al. (2017) and Terao et al. (2014). Other studies included asymptomatic persons whom were selected because of having a relative with RA,[3,4] being a twin,[2] or were blood bank donors.[6] In the latter no odds ratio was given on the association of smoking and ACPA in healthy individuals, however, since all required information was available we calculated the odds ratio from the data presented in the publication. In Terao et al. (2014) results were stratified

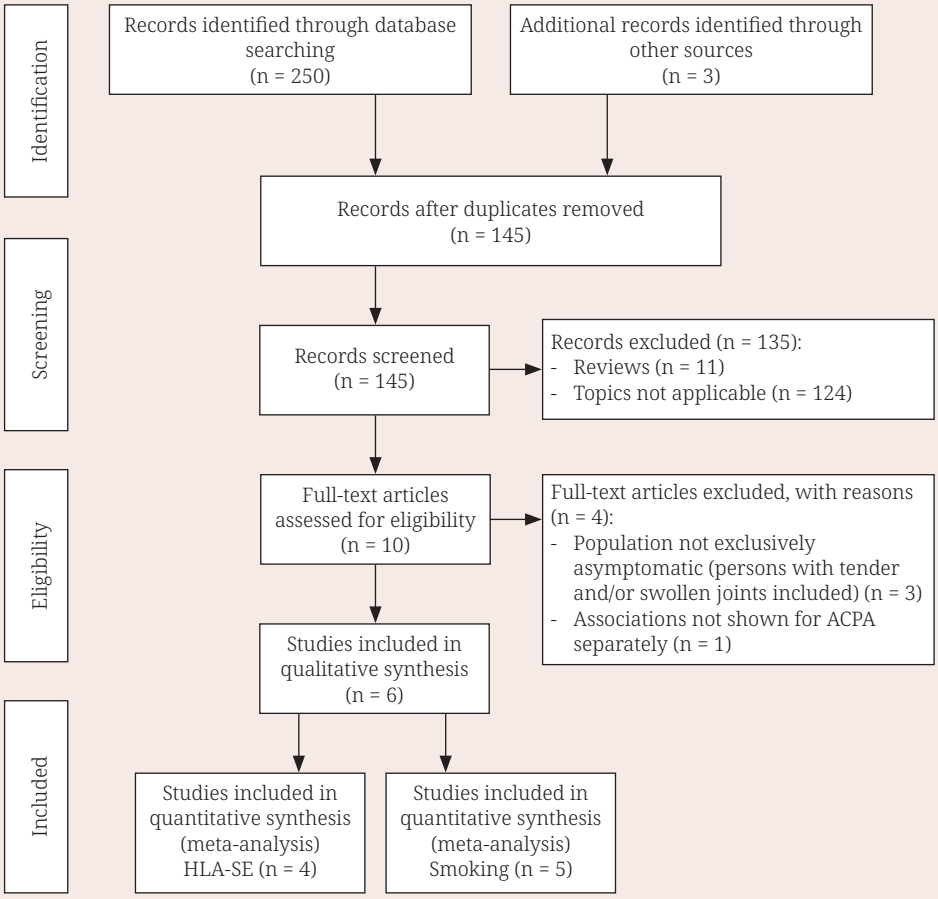
for gender; for the present meta-analyses we included results presented for women, however, results from the meta-analysis was similar when men were included in the analyses (data not shown).

Next, to eliminate possible inclusion bias, only the two studies that evaluated unselected individuals from the general population (van Zanten et al. (2017) and Terao et al. (2014)) were included in the meta-analysis. Then similar results were obtained, see Supplementary Figure 2.

Finally, since recent data in RA-patients indicated that smoking does not associate with ACPA as such, but rather with RF or autoantibodies in general,[8-12] and we wished to evaluate whether this is the same in the general population, we retrieved RF data, if present, from the identified studies, and performed additional meta-analyses. Two studies (Terao et al. (2014) and Ärlestig et al. (2011)) provided data on associations of HLA-SE and smoking with RF. Meta-analyses indicated that was no association was present between HLA-SE and RF (OR 0.97 (95% CI 0.70-1.35)), nor between smoking and RF (OR 0.84 (0.55-1.28); forest plots not shown).

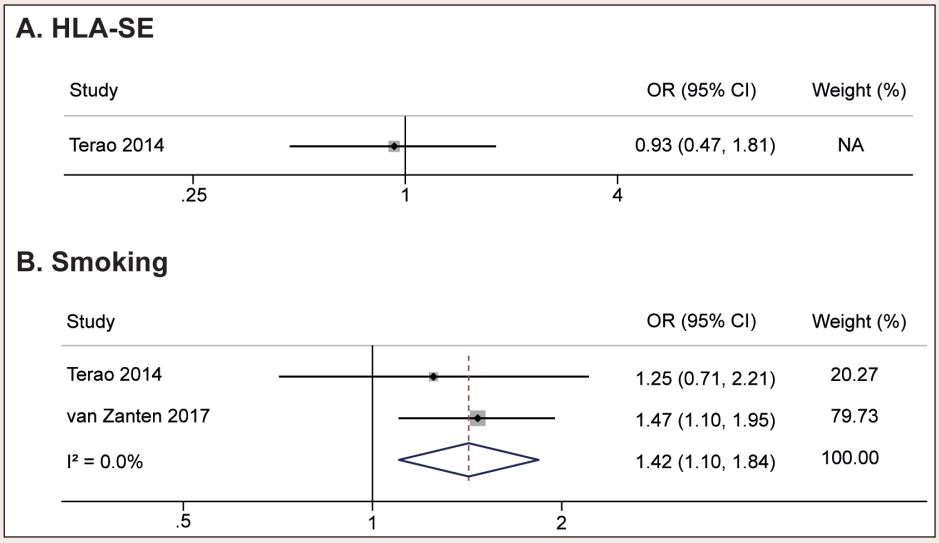
List of search terms that were used:
- SE smoking autoantibodies healthy (15 results)
- SE smoking autoantibodies asymptomatic (0 results)
- SE smoking autoantibodies preclinical (0 results)
- SE smoking ACPA healthy (8 results)
- SE ACPA healthy (32 results)
- shared epitope ACPA healthy (33 results)
- smoking ACPA healthy (32 results)
- smoking ACPA general population (16 results)
- shared epitope ACPA general population (10 results)
- smoking ACPA population-based (25 results)
- shared epitope ACPA population-based (15 results)
- smoking ACPA FDR (2 results)
- SE ACPA FDR (2 results)
- shared epitope ACPA FDR (2 results)
- SE smoking ACPA (58 results)

Supplementary Figure 1. Flowchart of literature review and article selection for meta-analyses on association of HLA-SE and smoking in the asymptomatic population.



Meta-analyses were performed in the 4 (HLA-SE) en 5 (smoking) included studies, respectively, without assessment of inclusion bias. Some of these studies did not select persons from the general asymptomatic population. Therefore sensitivity analyses with the population-based studies were performed, see Supplementary Figure 2.

Supplementary Figure 2. Meta-analyses on HLA-SE (A) and smoking (B) with presence of ACPA in unselected asymptomatic healthy population-based individuals



HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, OR: odds ratio, CI: confidence interval

Supplementary File 2 – Detailed description of methods

Leiden CSA cohort

Patients presenting with CSA to the Leiden rheumatology outpatient clinic between April 2012-September 2019 were studied. The cohort is described in detail previously.[13] Patients had recent-onset (<1 year) arthralgia of small joints and were, according to the clinical expertise and pattern recognition of the rheumatologist, at risk for progression to RA. Autoantibody status was largely unknown at inclusion as (in line with Dutch guidelines) general practitioners in the area of Leiden are discouraged to perform autoantibody tests. Inclusion in the CSA-cohort was therefore predominantly based on history taking and physical examination. Patients were excluded if clinical arthritis was already present, or if a different explanation for the joint pain was more likely. Patients were followed for at least 2 years for development of clinically apparent inflammatory arthritis (IA), defined as ≥1 swollen joints at physical examination by a rheumatologist, with scheduled follow-up visits at 4, 12 and 24 months. In case of an increase in symptoms or suspicion of clinical arthritis additional visits were planned. During follow-up treatment with disease-modifying anti-rheumatic drugs (DMARDs, including systemic or intra-articular corticosteroids) was not allowed, therefore patients participating in a randomized controlled trial investigating the effect of methotrexate in the phase of CSA (RCT; treat earlier) were excluded from the current study.

Baseline visit consisted of physical examination, blood sampling, questionnaires (including questions on current and past smoking) and an MRI. Presence of IgM RF (in house ELISA, cut-off >3.5 IU/mL) and IgG ACPA (anti-CCP2, Phadia, Nieuwegein, the Netherlands, cut-off >7 U/mL) was determined during routine laboratory measurements in all patients. Because of limited laboratory capacity not all patients were selected for additional anti-CarP and AAPA measurements. Patient selection for these tests was first based on availability of samples and presence of RF and/or ACPA at baseline. In total 189 patients were included for additional autoantibody measurements. First, patients positive for RF and/or ACPA at baseline with available serum samples were included (n=89, 45 progressing and 44 non-progressing patients). Then, autoantibody-negative patients with available samples that progressed to IA were additionally included (n=37). Finally, from the large group of autoantibody-negative patients that did not progress a random selection was made (n=63). Baseline characteristics of the randomly selected non-progressing autoantibody-negative patients were similar to that of the patients that were not selected (data not shown); suggesting that the selection is representative for this total group. The fact that anti-CarP and AAPA were only measured in a selection of autoantibody-negative patients

limits interpretation of predictive accuracy of these autoantibodies in the entire CSA-population. However, since no selection was made in RF-positive/ACPA-positive patients, analyses were repeated and validated with the Amsterdam cohort in this subgroup.

The study was approved by the local medical ethical committee and all participants gave written informed consent.

HLA-SE

HLA-SE status was derived from whole genome sequencing data. All patients were whole genome sequenced using the Illumina Global Screening Array (GSA). Standard quality control steps were performed using Plink v1.90;[14] individuals with a missingness of more than 2% were removed, as were variants with a missingness of over 2%, a minor allele frequency (MAF) below 0.01 or a Hardy-Weinberg equilibrium p-value below 0.0001. In order to prepare the genotype data for imputation of the HLA region we used the McCarthy program,[15] which checks for ambiguous SNPs with a MAF above 0.4 (only A/T & G/C SNPs), SNPs with a MAF deviating more than 0.2 from the HRC reference panel[16] and SNPs not in the aforementioned reference panel. We subsequently use the SNP2HLA tool with the T1DGC reference to impute the HLA region.[17] From these imputed data we extracted the HLA-SE, defined as the HLA-DRB1 variants *0101, *0102, *0401, *0404, *0405, *0408 and *1001.[18] Patients were deemed HLA-SE positive if 1 or 2 of the SE variants were present.

Anti-carbamylated and anti-acetylated antibodies

In serum, we determined the presence and levels of IgG anti-carbamylated and anti-acetylated protein antibodies (anti-CarP and AAPA, respectively). In-house ELISA was used for all measurements as described previously.[19] Briefly, plates were coated with carbamylated FCS and CCP1 acetylated lysine for measurements of anti-CarP and AAPA, respectively. To determine background signal, plates were additionally coated with non-modified antigens (non-modified FCS and CCP1 norleucine, respectively). Serum samples were diluted 1:50 and incubated. After washing, plates were subsequently incubated with HRP-labeled rabbit-anti-human IgG (Dako). HRP-activity was visualized with ABTS and measurements were expressed in arbitrary units per milliliter (aU/mL). On every plate a dilution standard was included to determine the linear part of the curve; standards from all plates were used in the analyses. The fourth standard, which is expected to be in the middle (and therefore linear part) of the curve, is further diluted and additionally included as a reference sample. Serum of healthy subjects (n=199) was used to determine the cut-off of all autoantibody measurements, which was calculated as the mean plus two times the standard deviation of healthy subjects. When the difference in optical density

(OD) between the non-modified antigens and the modified proteins was <0.1, the measurement was considered non-specific; non-specific measurements with values above the cut-off were considered negative. Inter-assay variation of in-house ELISAs was determined previously by reevaluation of ~10% of samples; measurements were highly correlated (Pearson's r ranges 0.96-0.97) and changes in positivity of the test were infrequent, see Supplementary Figure 4. Intra-assay variability was determined for anti-CarP by measurement of 3 samples 10 times, the mean coefficient of variation (CV, mean % (SD)) was 20.4 (6.8). Of note, although not absolute at the monoclonal- or polyclonal level, cross-reactivity of ACPA towards other post translationally modified proteins have been conclusively shown in different studies,[20,21] and hence should be regarded as anti-modified protein antibody-reactivities.

Amsterdam arthralgia cohort

Analyses evaluating progression to IA were stratified for ACPA-status and results from the ACPA-positive subgroup were studied in meta-analyses with the results from two other ACPA-positive cohorts. The first cohort concerned arthralgia patients from Amsterdam, as described in van de Stadt et al.[22] As this study evaluated ACPA- and/or RF-positive patients, raw data from ACPA-positive individuals was obtained for this study. Data on smoking was obtained by history taking. Presence of HLA-SE was inferred from HLA-DQA1, HLA-DQB1 haplotypes using strong linkage disequilibrium with HLA-DRB1 alleles in Caucasians.[23] Patients were positive for HLA-SE if 1 or 2 copies of the HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410 or *1001 alleles were present. Presence of IgM RF and ACPA were determined with in-house ELISA (cut-off >30 IU/mL) and aCCP ELISA (Axis Shield; cut-off >5 aU/mL), respectively. Presence of anti-CarP was determined with in-house ELISA similar to methods used in the CSA cohort.[19] Baseline serum samples were obtained to determine presence of IgG AAPA simultaneously with Leiden CSA-samples.

Leeds ACPA-positive cohort

The second cohort consists of ACPA-positive patients with non-specific musculoskeletal symptoms from the Leeds cohort, as described by Rakieh et al.[24] Since only ACPA-positive patients were studied, results obtained from the literature were sufficient for inclusion in the meta-analyses; raw data was not evaluated. Also here smoking status was obtained through history taking. Presence of HLA-SE was determined by PCR amplification with sequence-specific primers.[25] Patients were positive for HLA-SE if 1 or 2 copies of alleles in the HLA-DRB1*01, *04 and *10 locus were present. Presence of IgM RF (initial cut-off >40 IU/mL, later >20 IU/mL) and ACPA (anti-CCP2, immunocap assay, Phadia; initial cut-off >7 IU/mL, later >2.99 IU/mL) was determined. No serum samples were obtained from this cohort and the presence of anti-CarP and AAPA was not determined.

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Supplementary Table 1. Baseline characteristics

	CSA cohort Leiden			Arthralgia cohort Amsterdam
	All patients (n=577)	*ACPA-positive (n=77)*	*ACPA-negative (n=500)*	*ACPA-positive (n=244)*
Female, n (%)	451 (78.2)	61 (79.2)	390 (78.0)	186 (76.2)
Age in years, mean (SD)	43.7 (12.6)	48.1 (12.0)	43.0 (12.5)	48.4 (11.4)
Symptom duration in weeks, median (IQR)	20 (9-46)	25 (13-53)	19 (9-43)	52 (28-104)
53-TJC, median (IQR)	5 (2-10)	3 (2-7)	5 (2-10)	0 (0-3)
Increased CRP, n (%)	126 (22.0)	23 (30.3)	103 (20.7)	35 (14.5)
RF positivity, n (%)	113 (19.6)	59 (76.6)	54 (10.8)	108 (44.3)
ACPA positivity, n (%)	77 (13.3)	NA	NA	NA

Data on HLA-SE and smoking was available in the CSA cohort in 522 and 500 patients, respectively. In Amsterdam in 133 and 243 patients, respectively.
CSA: clinically suspect arthralgia, ACPA: anti-citrullinated protein antibody, SD: standard deviation, IQR: interquartile range, TJC: tender joint count, RF: rheumatoid factor, CRP: c-reactive protein

Supplementary Table 2. Associations of HLA-SE and smoking with ACPA-positivity stratified for RF, in patients newly presenting with CSA

		ACPA positive, n (%)	ACPA negative, n (%)	OR (95% CI)	p-value
RF-positive patients					
HLA-SE	Absent	22 (42)	32 (64)	Reference	--
	Present	31 (59)	18 (36)	2.51 (1.13-5.55)	0.024
HLA-SE	0	22 (42)	32 (64)	Reference	--
	1	23 (43)	16 (32)	2.09 (0.91-4.83)	0.084
	2	8 (15)	2 (4)	5.82 (1.13-30.05)	0.036
Smoking	Never	13 (26)	23 (49)	Reference	--
	Ever	37 (74)	24 (51)	2.73 (1.16-6.40)	0.021
Smoking	Never	13 (26)	23 (49)	Reference	--
	Ex-smoker	22 (44)	21 (45)	1.85 (0.75-4.58)	0.18
	Current smoker	15 (30)	3 (6)	8.85 (2.15-36.38)	0.003
RF-negative patients					
HLA-SE	Absent	5 (31)	227 (56)	Reference	--
	Present	11 (69)	176 (44)	2.84 (0.97-8.32)	0.057
HLA-SE	0	5 (31)	227 (56)	Reference	--
	1	8 (50)	145 (36)	2.51 (0.80-7.81)	0.11
	2	3 (19)	31 (8)	4.39 (1.0-19.30)	0.050
Smoking	Never	2 (14)	162 (42)	Reference	--
	Ever	12 (86)	227 (58)	4.28 (0.95-19.39)	0.059
Smoking	Never	2 (14)	162 (42)	Reference	--
	Ex-smoker	6 (43)	140 (36)	3.47 (0.69-17.48)	0.13
	Current smoker	6 (43)	87 (22)	5.59 (1.10-28.27)	0.038

HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, RF: rheumatoid factor, CSA: clinically suspect arthralgia, OR: odds ratio, CI: confidence interval

Supplementary Table 3. Associations of HLA-SE and smoking with ACPA-level in ACPA-positive patients evaluated with Mann-Whitney U tests and logistic regression

HLA-SE positive	**HLA-SE negative**	**Mann-Whitney U**	**Logistic regression**
Median ACPA level (IQR)	*Median ACPA level (IQR)*	*p-value*	*OR (95% CI), p-value*
236 (72-340)	144 (32-340)	0.12	1.002 (0.999-1.006), 0.18

Ever smoking	**Never smoking**	**Mann-Whitney U**	**Logistic regression**
Median ACPA level (IQR)	*Median ACPA level (IQR)*	*p-value*	*OR (95% CI), p-value*
229 (64-340)	222 (52-340)	0.89	1.000 (0.996-1.004), 0.99

Odds ratios indicate effect measures for every unit increase in ACPA-level.
HLA-SE: shared epitope, ACPA: anti-citrullinated protein antibody, IQR: interquartile range, OR: odds ratio, CI: confidence interval

Supplementary Table 4. Associations of HLA-SE with development of inflammatory arthritis in patients newly presenting with CSA; hazard ratios corresponding to Figure 2 of the main article

		IA, n (%)	No IA, n (%)	HR (95% CI)	p-value
All patients					
HLA-SE	Absent	39 (42)	247 (58)	Reference	--
	Present	55 (59)	181 (42)	1.86 (1.23-2.82)	0.003
HLA-SE	0	39 (42)	247 (58)	Reference	--
	1	41 (44)	151 (35)	1.65 (1.06-2.56)	0.027
	2	14 (15)	30 (7)	3.03 (1.64-5.61)	<0.001
ACPA positive subgroup					
HLA-SE	Absent	15 (37)	12 (43)	Reference	--
	Present	26 (63)	16 (57)	1.29 (0.67-2.47)	0.44
HLA-SE	0	15 (37)	12 (43)	Reference	--
	1	17 (42)	14 (50)	1.05 (0.52-2.13)	0.90
	2	9 (22)	2 (7)	2.32 (1.00-5.41)	0.051
ACPA negative subgroup					
HLA-SE	Absent	24 (45)	235 (59)	Reference	--
	Present	29 (55)	165 (41)	1.71 (0.99-2.96)	0.055
HLA-SE	0	24 (45)	235 (59)	Reference	--
	1	24 (45)	137 (34)	1.66 (0.94-2.94)	0.083
	2	5 (9)	28 (7)	2.00 (0.76-5.28)	0.16

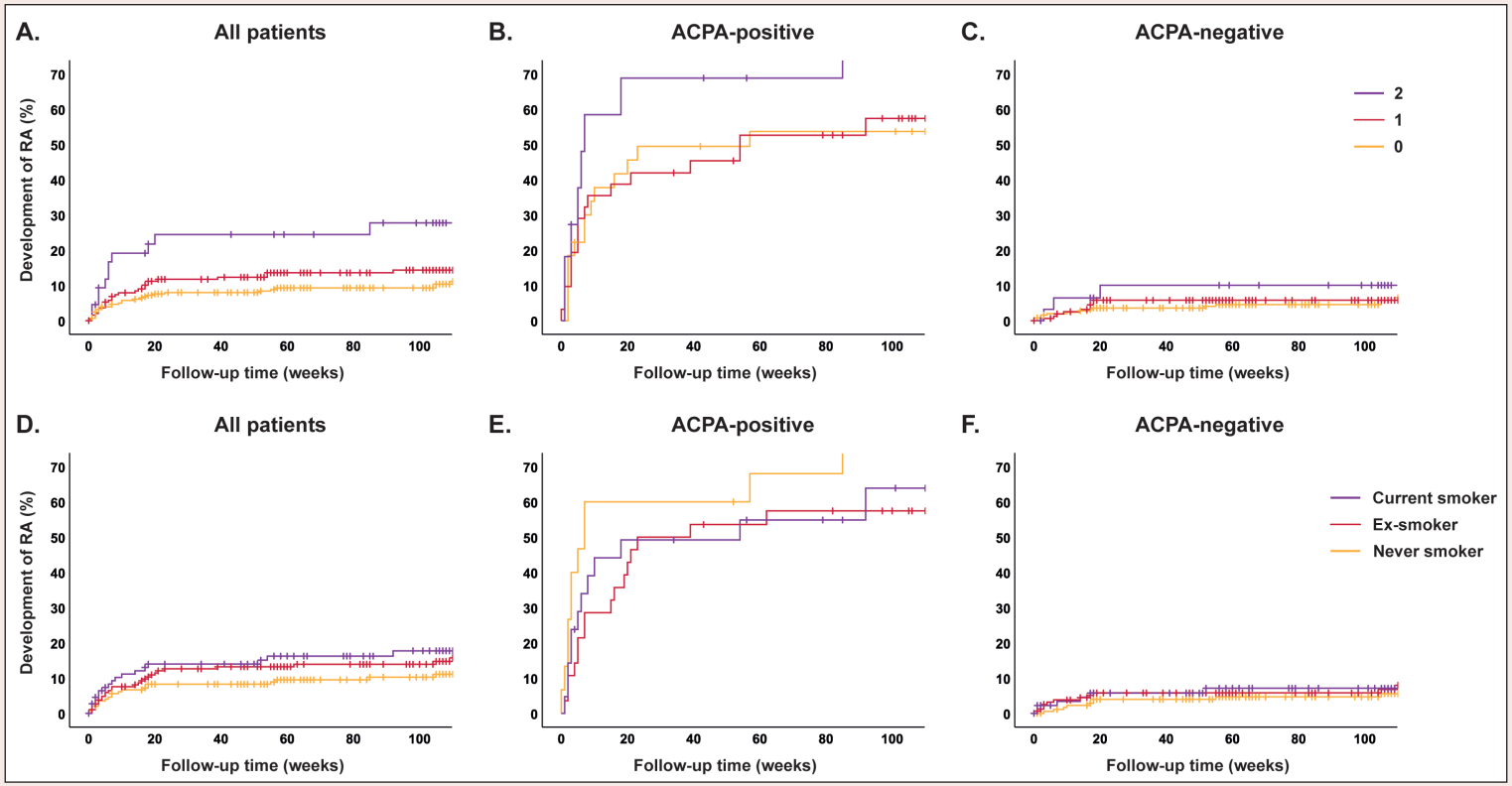
HLA-SE: shared epitope, CSA: clinically suspect arthralgia, IA: inflammatory arthritis, HR: hazard ratio, CI: confidence interval, ACPA: anti-citrullinated protein antibody

Supplementary Table 5. Associations of smoking with development of inflammatory arthritis in patients newly presenting with CSA; hazard ratios corresponding to Figure 3 of the main article

		IA, n (%)	No IA, n (%)	HR (95% CI)	p-value
All patients					
Smoking	Never	29 (32)	171 (42)	Reference	--
	Ever	63 (69)	237 (58)	1.40 (0.90-2.18)	0.14
Smoking	Never	29 (32)	171 (42)	Reference	--
	Ex-smoker	38 (41)	151 (37)	1.25 (0.76-2.06)	0.37
	Current smoker	25 (27)	86 (21)	1.66 (0.97-2.83)	0.065
ACPA positive subgroup					
Smoking	Never	11 (28)	4 (17)	Reference	--
	Ever	29 (73)	20 (83)	0.59 (0.29-1.18)	0.13
Smoking	Never	11 (28)	4 (17)	Reference	--
	Ex-smoker	17 (43)	11 (46)	0.55 (0.26-1.19)	0.13
	Current smoker	12 (30)	9 (38)	0.64 (0.28-1.45)	0.28
ACPA negative subgroup					
Smoking	Never	18 (35)	167 (44)	Reference	--
	Ever	34 (65)	217 (57)	1.30 (0.73-2.33)	0.37
Smoking	Never	18 (35)	167 (44)	Reference	--
	Ex-smoker	21 (40)	140 (37)	1.17 (0.61-2.24)	0.64
	Current smoker	13 (25)	77 (20)	1.56 (0.76-3.18)	0.23

CSA: clinically suspect arthralgia, IA: inflammatory arthritis, HR: hazard ratio, CI: confidence interval, ACPA: anti-citrullinated protein antibody

Supplementary Figure 3. Associations of HLA-SE (A-C) and smoking (D-F) with progression from CSA to RA



RA is defined as fulfilment of the 1987 and/or 2010 criteria at the time of clinically apparent inflammatory arthritis development.
Corresponding hazard ratios, with 0 HLA-SE alleles as reference category were: (A) HR 1.42 (95% CI 0.85-2.38) and HR 2.97 (1.48-5.96) for 1 and 2 HLA-SE alleles respectively, (B) HR 1.05 (0.52-2.13) and HR 2.08 (0.87-5.00), and (C) HR 1.16 (0.53-2.53) and HR 1.87 (0.54-6.51).
Corresponding hazard ratios, with never smoker as reference category were: (D) HR 1.44 (0.81-2.56) and HR 1.81 (0.97-3.39) for ex-smoker and current smoker respectively, (E) HR 0.55 (0.26-1.19) and HR 0.64 (0.28-1.45), and (F) HR 1.42 (0.59-3.43) and HR 1.66 (0.62-4.46).
HLA-SE: shared epitope, CSA: clinically suspect arthralgia, RA: rheumatoid arthritis, ACPA: anti-citrullinated protein antibody, HR: hazard ratio, CI: confidence interval

Supplementary Table 6. Associations of HLA-SE and smoking with presence of RF, anti-CarP and AAPA in patients newly presenting with CSA

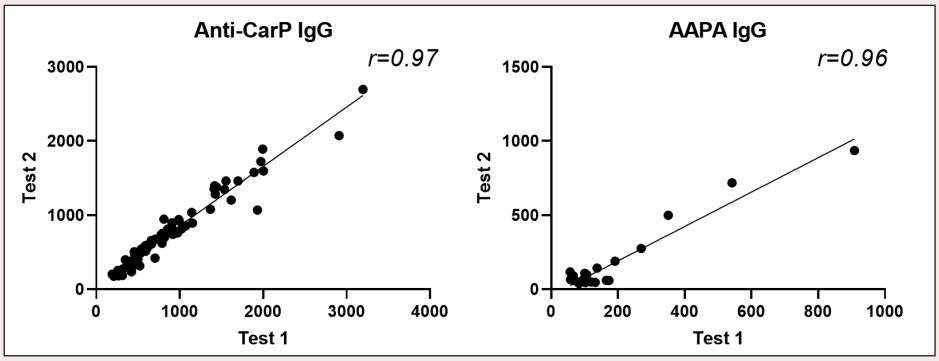
RF	Univariable OR (95% CI)	p-value	Multivariable[a] OR (95% CI)	p-value
HLA-SE present	1.13 (0.73-1.73)	0.59	0.71 (0.41-1.22)	0.21
Ever-smoker	1.16 (0.74-1.84)	0.52	0.70 (0.40-1.23)	0.22

Anti-CarP	Univariable OR (95% CI)	p-value	Multivariable[a] OR (95% CI)	p-value
HLA-SE present	1.63 (0.59-4.50)	0.34	1.31 (0.42-4.07)	0.65
Ever-smoker	1.05 (0.37-2.95)	0.93	0.58 (0.17-2.01)	0.39

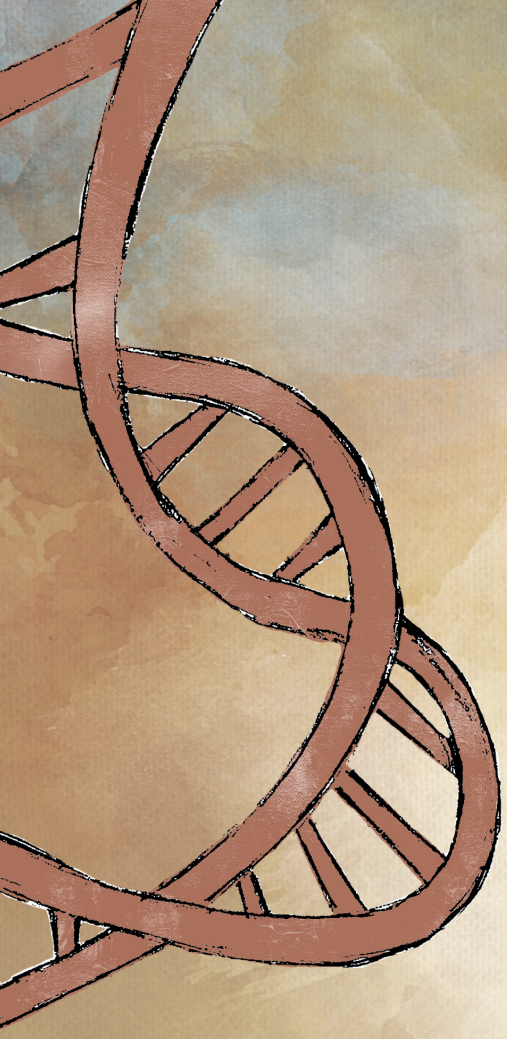
AAPA	Univariable OR (95% CI)	p-value	Multivariable[a] OR (95% CI)	p-value
HLA-SE present	1.41 (0.65-3.05)	0.39	1.15 (0.47-2.82)	0.76
Ever-smoker	0.58 (0.26-1.31)	0.19	0.28 (0.098-0.80)	0.018

[a] Corrected for presence of ACPA
HLA-SE: shared epitope, RF: rheumatoid factor, anti-CarP: anti-carbamylated protein antibody, AAPA: anti-acetylated protein antibody, CSA: clinically suspect arthralgia, OR: odds ratio, CI: confidence interval, ACPA: anti-citrullinated protein antibody

Supplementary Figure 4. Inter-assay variation of in-house ELISAs

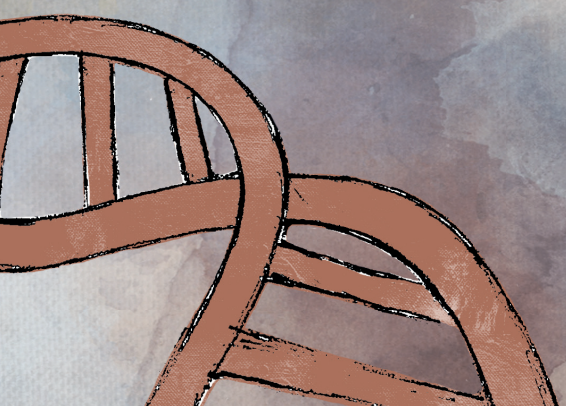


Inter-assay variation resulted in changes in positivity of the test infrequently: anti-CarP IgG 3.9%, AAPA IgG 4.2%.
anti-CarP: anti-carbamylated protein antibody, AAPA: anti-acetylated protein antibody



8

Summary and discussion



In this thesis two main aims were addressed. It has long been established that early treatment of rheumatoid arthritis (RA) improves disease outcomes. In **Part I** of this thesis we therefore further investigated the early detection of at-risk individuals by studying a large cohort of patients with clinically suspect arthralgia (CSA). We explored the value of two easy clinical tests, their potential to detect underlying inflammatory processes and to predict disease progression. In addition we investigated the presence of subclinical synovitis on imaging as starting point for treatment with disease modifying anti-rheumatic drugs (DMARDs) and the value of magnetic resonance imaging (MRI) detected erosions as new predictor for RA-development. In **Part II** of this thesis we aimed to determine which disease processes are involved in the different phases of RA-development. Knowledge on disease pathogenesis and timing of influencing factors can help to better target treatment during RA-development. We therefore evaluated whether autoantibody-response maturation occurred during the phase of CSA, and investigated the timing of genetic risk factor human leukocyte antigen-shared epitope (HLA-SE) and environmental risk factor smoking during the development of autoantibody-positive disease.

Part I – Prediction and early detection of rheumatoid arthritis

Summary Chapter 2 and 3

The value of imaging in the prediction of RA has often been investigated. Subclinical inflammation can be detected even before the occurrence of clinically detectable arthritis and has been shown to predict disease progression.[1,2] However, imaging modalities as ultrasonography (US) and MRI are costly, time consuming and not always available. Moreover, it was hypothesized that subclinical features might underly clinical manifestations that are considered risk factors for development of RA. In **Chapter 2** we therefore investigated difficulties making a fist, one of the factors comprising the EULAR definition of arthralgia suspicious for progression to RA.[3] Although fist problems are considered a risk factor for RA-development in patients presenting with CSA, its predictive value and underlying cause were unclear. Difficulties making a fist was assessed in two ways: 1) fist closure was evaluated by visual inspection of the ability to completely close the fist, all fingertips touching the palm, 2) fist strength was determined by the patient squeezing the assessor's fingers. Incomplete fist closure and a decreased fist strength were both independently associated with progression to clinically apparent inflammatory arthritis (IA), though incomplete fist closure had a higher predictive value and better reliability. Fist problems associated significantly with flexor tenosynovitis; incomplete fist closure associated predominantly with flexor tenosynovitis of metacarpophalangeal (MCP)

joints, whereas decreased fist strength more strongly related to flexor tenosynovitis of the wrist. These results indicate that difficulties making a fist, and predominantly fist closure, is easily assessable in clinical practice and can provide information on both risk assessment for disease progression as well as underlying flexor tenosynovitis.

In addition to fist problems, the value of another simple clinical test in CSA was studied. Historically, the squeeze test, i.e. compression across the knuckles of MCP and metatarsophalangeal (MTP) joints, was used to assess presence of synovitis.[4] In early arthritis a positive squeeze test was indeed shown to associate with presence of synovitis in MCP- and MTP-joints, and even with local MRI-detected inflammation.[5] In the phase of CSA the squeeze test is considered a risk factor for development of RA, as it is also incorporated in the EULAR definition of arthralgia suspicious for progression to RA. It was therefore hypothesized that a positive squeeze test in CSA, in absence of clinical arthritis, might associate with presence of subclinical inflammation; this was investigated in **Chapter 3**. It was shown that >50% of CSA-patients had positive squeeze test in MCP- or MTP-joints, and that a positive test independently associated with local subclinical synovitis with an OR of 2. However, the sensitivity of the test was only 44%, indicating that subclinical synovitis is also often missed. In addition, a positive squeeze test in CSA was not independently associated with progression to IA. Nevertheless, despite its lack in predictive value, the squeeze test is a simple and quick test that can be used to obtain a first indication on presence of subclinical synovitis.

Considerations from Chapter 2 and 3

Chapter 2 and 3 describe two closely related tests that are part of the physical examination in clinical practice; difficulties making a fist and the squeeze test, both performed with different hand positioning. During development of the EULAR definition, experts indicated that both tests contributed to the recognition of CSA, and their independent contribution was confirmed in statistical analyses.[3] In **Chapter 2 and 3** we aimed to increase our understanding of these tests, and discovered that they both associate with different inflammatory features; fist problems with tenosynovitis and the squeeze test with synovitis. This confirms the notion that both tests are in fact different and correlate with different types of subclinical inflammatory features. This suggests that both tests could be of value in the recognition of CSA in clinical practice.

Together, assessment of fist problems and the squeeze test help provide a first impression of underlying subclinical inflammation. Importantly, both studies were performed in a population of CSA-patients. The results can therefore not be generalized to other populations without further research. Primary care is a population where both tests might be of value in establishing a first risk assessment,

since general physicians acknowledge the importance but also the difficulty of differentiating inflammatory diseases from other musculoskeletal problems.[6,7] However, the predictive value of a test is dependent on the prevalence of disease in a population, i.e. the pre-test probability. The incidence of RA, and presumably also the prevalence of subclinical inflammation in primary care is low. Therefore it is likely that the predictive value of these tests is also lower in primary care than in CSA. Nevertheless, since test characteristics are unaffected by prevalence, the sensitivity and specificity will remain the same in primary care.

Summary Chapter 4

When presence of subclinical inflammation in at-risk populations is confirmed with imaging, treatment is sometimes considered, even in absence of clinical arthritis. Studies have shown that an increasing number of rheumatologists consider or initiate DMARD-treatment in patients with autoantibody-positive arthralgia,[8] their choices guided by US findings and presence of subclinical inflammation.[9] Indeed, subclinical inflammation can precede development of IA, but subclinical inflammation and symptoms also often spontaneously resolve.[10] In **Chapter 4** we therefore addressed the value of subclinical synovitis, its potential as starting point for DMARD-treatment and its potential for overtreatment. We studied three arthralgia cohorts in which the presence of subclinical synovitis was determined at baseline by either US or MRI. All patients were followed for one year for development of IA, during which DMARD-treatment (including corticosteroids) was not allowed. In anti-citrullinated protein antibody (ACPA) positive patients with subclinical synovitis 50-68% of patients did not develop IA, in ACPA-negative patients 66-89% did not progress. Even in patients with additionally ≥6 points on the 2010 EULAR classification criteria for RA, false positive rates remained considerable (≥37%). Results also remained similar when more stringent definitions of subclinical synovitis were used in sensitivity analyses. These findings indicate that DMARD-treatment in arthralgia-patients with subclinical synovitis would lead to considerable overtreatment.

Considerations from Chapter 4

In **Chapter 4** both MRI and US were used. It has been shown that MRI has a higher sensitivity than US.[11] Nevertheless, false-positive rates in all three cohorts were high, and the use of different imaging modalities with varying sensitivity has therefore unlikely influenced conclusions from this study. False positive rates might be further decreased by additional evaluation of other inflammatory features, e.g. tenosynovitis and/or BME. The latter can only be visualized by MRI, and the predictive value independent from tenosynovitis is limited.[2] Tenosynovitis is detectable by both imaging modalities, and the predictive value is highest of all inflammatory features.[2,12] It would therefore be valuable to repeat this study with subclinical tenosynovitis as

potential starting point for DMARD-treatment.

Summary Chapter 5

Subclinical inflammation as measured in **Chapter 2, 3 and 4**, even combined with ACPA-status, is insufficient for reliable identification of patients that progress to IA. Other imaging factors potentially increase the prognostic value of subclinical inflammation. Bone erosions are a hallmark of RA, and even RA-specific MRI-detected erosions have been established; these erosions were present in patients with early RA, but not in patients with other arthritides.[13] MRI is sensitive in detection of bone erosions, even in symptom-free persons[14] and in patients with CSA small MRI-detected erosions are detectable. In **Chapter 5** we investigated MRI-detected erosions in the phase of CSA. We determined the predictive value of MRI-detected erosions for development of IA, and evaluated whether the prognostic value of MRI-detected subclinical inflammation could be improved by evaluation of MRI-detected erosions. Any MRI-erosion, defined as erosions that were present in <5% of symptom-free persons in the same bone and age category, was present in 20% of CSA-patients. Presence of these erosions was not associated with IA-development. Erosion characteristics previously reported as specific for RA (grade ≥2 erosions, erosions in MTP5 and erosions in MTP1 in persons aged <40) were rarely seen in CSA-patients, and their presence was not associated with IA-development. When MRI-detected erosions were considered in addition to MRI-detected subclinical inflammation, the area under the curve (AUC) did not improve, and the prognostic accuracy decreased as shown by a net reclassification index (NRI) of -5.8; adding data on MRI-erosions resulted in a high number of false-positive predictions. Since erosions mainly occur early in ACPA-positive disease,[15-17] MRI-erosions were also evaluated in ACPA-positive and ACPA-negative CSA-patients separately. In neither subset MRI-detected erosions were predictive for development of IA. However, the median erosion score in ACPA-positive patients was significantly higher than in ACPA-negative patients. Notably, this difference was only seen in patients with subclinical inflammation; ACPA-positive patients without subclinical inflammation did not have a higher erosion score than ACPA-negative patients without subclinical inflammation.

Considerations from Chapter 5

Findings in **Chapter 5** are supported by previous studies in undifferentiated arthritis (UA). The prevalence of RA-specific erosions was similar between UA- and CSA-patients,[18] which is in line with the finding that erosion-scores in CSA-patients did not increase during progression to IA.[19] Additionally, in UA-patients erosions also lacked predictive value for development of RA.[18] Nevertheless, more erosions were present in CSA compared to symptom-free controls. Even though the exact mechanism is unclear, the erosions in CSA might reflect previous subclinical inflammation that, due

to lack of other stimuli, spontaneously resolved. Intriguingly, a significantly higher number of erosions was seen in ACPA-positive CSA-patients, though only when subclinical inflammation was present. This supports the finding that development of erosions in ACPA-positive CSA is mediated by subclinical inflammation, as shown previously.[15] Potentially also a direct effect of ACPA on osteoclastogenesis and bone resorption exists, as indicated by a study from Harre et al.[20] However, since the number of erosions was not increased in CSA-patients with only ACPA (i.e. without subclinical inflammation), our data could not support this finding, Further research unravelling the mechanism between ACPA and development of erosions is needed. Because even though small MRI-detected erosions cannot be used as predictor for imminent RA, this study indicated that erosions already occur in the phase of CSA. Knowledge on the interplay between pro-inflammatory factors and ACPA in this process might direct future prevention of bone damage early in the disease.

Overall considerations from Part I

All longitudinal studies in **Part I** of this thesis were potentially influenced by the Treat Earlier trial, a randomized controlled trial (RCT) carried out in CSA-patients.[21] From April 2015 until September 2019 CSA-patients with MRI-detected subclinical inflammation could be included in this trial, in which the effect of Methotrexate (MTX) was studied. Baseline characteristics of included patients could be used for cross-sectional analyses within the CSA-cohort. However, since the outcome of the CSA-cohort (development of IA) was potentially influenced by the 50% chance of DMARD-treatment in patients participating in this trial, these patients had to be excluded from longitudinal analyses. Excluding part of the patients with subclinical inflammation, a known risk factor for development of RA, might have influenced the associations of investigated predictors. Robustness of our findings has been investigated by stratification for subclinical inflammation, evaluation of only patients included in the CSA-cohort before the start of the trial and/or validation in other at-risk cohorts; these analyses consistently indicated similar results. It is therefore considered unlikely that the exclusion of part of the patients in longitudinal analyses caused incorrect results.

The predictors investigated in this part of the thesis were insufficient in establishing a CSA-population that would progress to clinical arthritis with certainty. MRI-detected erosions were not helpful and even increased the number of false-positive predictions. Subclinical synovitis was present in a high number of CSA-patients that did not progress to IA, and the squeeze test as proxy of subclinical synovitis did not yield predictive value. Likewise, despite tenosynovitis having the highest predictive value of all investigated features, not all CSA-patients with subclinical tenosynovitis, or with difficulties making a fist as proxy thereof, progressed to IA. It seems necessary to combine multiple predictors to obtain high predictive values. Indeed, prediction

rules including known clinical and immunological predictors,[22] combinations of MRI features,[12] or even both[23] have been shown to increase the AUC and positive predictive values (PPVs). Newly discovered imaging features might further improve these models. The discovery of other juxta-articular features as intermetatarsal bursitis (IMB), which has been associated with early RA[24] and conferred risk for arthritis development in CSA,[25] might be used for this purpose.

In addition, imaging techniques continuously improve, which may further enhance implementation of imaging in clinical practice. Shorter MRI scanning protocols without contrast-enhancement using modified Dixon sequences provide similar results as MRI sequences used in this thesis, though less costly and more patient-friendly.[26] Additionally, a decrease in workload for physicians and researchers, as well as an increase in faster and more consistent scoring methods is pursued by investigating possibilities of automated scoring methods with artificial intelligence (AI).

Nevertheless, despite improvements of imaging methods and discovery of new predictive features, advanced imaging modalities are not always (directly) available. It remains important to keep investigating associations of clinical features with (new) predictive imaging features, such as the squeeze test and difficulties making a fist which proved useful in this thesis. Their value should be investigated in future prediction models, which can improve applicability of prediction models in clinical practice and optimize a first risk assessment. Since presence of subclinical extensor peritendinitis of MCP-joints has been shown to have a high predictive value,[12] determining a clinical proxy for this feature might prove valuable in clinical practice.

Autoantibodies are among the first factors thought of in prediction of RA. ACPA and RF are among the strongest predictors for development of RA, and also play an important role in the 2010 EULAR classification criteria for RA.[27] Nevertheless, in up to 50% of RA-patients autoantibodies are absent. Even though the long-term outcomes of autoantibody-negative patients are less severe, clinical presentation and functional limitations are just as severe as in autoantibody-positive patients.[28] This underlines the importance of identifying not only early autoantibody-positive RA, but also early autoantibody-negative RA. At the moment most at-risk populations are composed of (arthralgia-)subjects with ACPA and/or RF, or relatives of RA-patients (e.g. first-degree relatives (FDR)). The CSA-cohort consists of a unique at-risk population, its inclusion based on clinical presentation rather than autoantibody status. While this cohort is useful in identification of predictors for autoantibody-negative RA, large cohorts for validation are lacking. Even though the recognition of autoantibody-negative RA is more complicated by the absence of known immunological risk factors, it deserves

more attention in pre-clinical research.

Even when existing prediction rules are improved, it is likely that part of at-risk patients will still not progress to arthritis, despite presentation with one or more known predictors. This complicates early preventive treatment, as it will partly result in overtreatment. Overtreatment is not without consequences; DMARD-treatment is expensive and may cause side effects. However, as functional limitations in patients with CSA that later progress to IA are already as severe as at the moment of arthritis development,[29] treatment may significantly benefit some at-risk patients. To what extent overtreatment is acceptable is an important topic of discussion. Apart from the risk for overtreatment, preventive treatment also complicates new studies in the pre-arthritis phase. The natural disease course that is presently studied in observational cohorts is then influenced by potential treatment responses in part of the patients. Moreover, ethical concerns may arise when initiating clinical trials investigating new preventive treatment in which part of the patients would receive placebo treatment. Lastly, the effectiveness of treatment in prevention of RA is not clear yet,[30-32] and currently still investigated in several trials.[21,33-35] It is therefore recommended to await clinical trial results and optimize prediction rules before starting DMARD-treatment in populations where clinical arthritis is not yet present.

Part II – Pathogenesis of rheumatoid arthritis

Adequate identification of patients at risk for development of RA can be improved by understanding the disease pathogenesis. Knowledge on disease processes and timing of contributing factors during initiation and progression of the disease help to better target treatment in pre-clinical stages, ultimately preventing RA.

Summary Chapter 6

Autoantibody development and response maturation, defined as an increase in number of autoantibodies and autoantibody levels, precede the development of RA.[36-38] It was unknown whether autoantibody-response maturation occurred in the phase of CSA, or whether the response was already fully matured at the onset of symptoms. In **Chapter 6** we therefore evaluated the presence and levels of autoantibodies in CSA at two timepoints; in patients that progressed to IA samples were taken at baseline and at the moment of IA-development, in patients that did not progress samples were taken at baseline and after two years. If maturation of the autoantibody-response played a role in disease progression, maturation was expected to be only present in CSA-patients that developed IA. We analyzed three autoantibodies (ACPA, anti-carbamylated protein antibodies (anti-CarP) and anti-

acetylated protein antibodies (AAPA)) in three different isotypes (IgM, IgG and IgA). Patients without any autoantibody at baseline rarely seroconverted to positive during follow-up. In patients with ≥1 autoantibody (out of nine) at baseline the median number of autoantibodies was 1, and an increase in number of autoantibodies was infrequent. Autoantibody levels did not significantly change over time. These findings were similar between patients that progressed to IA and patients that did not progress. We therefore concluded that autoantibody-response maturation was not responsible for the final hit in development of clinical arthritis. However, when the outcome RA was used, i.e. fulfilment of the 1987 and/or 2010 ACR/EULAR criteria[27,39] at the moment of IA-development, patients who did not progress showed a decrease in median number of autoantibodies over time. Possibly other factors involved in the continuation of the autoantibody-response were lacking in these patients.

Considerations from Chapter 6

Due to laboratory capacity autoantibodies at two time-points could only be measured in part of CSA-patients. This predominantly affected the selection of non-progressing CSA-patients that tested negative for RF and/or ACPA during routine laboratory measurements at baseline; from this group a random sample was studied. Preferably the entire CSA-population would have been studied. However, since baseline characteristics of included and excluded patients were similar, it is unlikely that replication of this study in the entire CSA-population would yield different results. Ideally, population-based studies are performed to confirm that autoantibody-response maturation occurs predominantly in the asymptomatic, and not in the symptomatic phase of RA-development.

Summary Chapter 7

In **Chapter 7** we focused on the role and timing of the two most prominent genetic and environmental risk factors for development of RA; HLA-SE and smoking. Their association with RA is widely acknowledged, though it is unknown at which disease stage they exert their effect. To investigate a potential role of HLA-SE and smoking in autoantibody-development we studied literature on associations of HLA-SE and smoking with presence of ACPA in the asymptomatic population. Meta-analyses revealed that smoking, but not HLA-SE, was associated with ACPA-positivity in asymptomatic individuals. At presentation with symptoms (CSA-onset) both HLA-SE and smoking associated with presence of ACPA. Though previous studies showed gene-environment interactions for development of ACPA,[40,41] in CSA the association of smoking with ACPA was not dependent on presence of HLA-SE, and no significant interaction between HLA-SE and smoking was found. Likewise, previous findings in RA indicating that smoking was not associated with ACPA, but with RF or autoantibodies in general,[42,43] could not be replicated in meta-analyses of

asymptomatic populations or in CSA-patients. During follow-up HLA-SE associated with progression to IA in the total CSA-population, as well as in the ACPA-positive subset as indicated by meta-analyses in three arthralgia-cohorts. Smoking was not associated with IA-development, not in the total CSA-population, after ACPA-stratification or in meta-analyses with other cohorts. Together, results from this study imply that smoking is involved in autoantibody-development, and possibly symptom-development, but not with further progression to IA. The HLA-SE is not involved in autoantibody-development, potentially plays a role in autoantibody-maturation and symptom-development, and associates with IA-development.

Considerations from Chapter 7

Importantly, conclusions from **Chapter 7** are partly based on results from cross-sectional data. Associations of smoking and HLA-SE with autoantibody-development were investigated in cross-sectional studies of asymptomatic populations. However, we believe that autoantibody-development is the first step towards development of autoantibody-positive RA. Therefore it is likely that these cross-sectional data accurately reflect the roles of smoking and HLA-SE in autoantibody-development. Associations of smoking and HLA-SE with autoantibody-response maturation and symptom-development have been based on data obtained at CSA-onset. Though different studies support findings of smoking having a role in progression to CSA,[44] and HLA-SE being involved in autoantibody-response maturation,[45] longitudinal data are needed to confirm these findings. Ideally, population-based studies following individuals from an asymptomatic stage towards development of RA are performed. Such studies are complicated by the low prevalence of RA in the general population; an excessive number of subjects is needed to detect even a few that eventually develop RA.

In **Chapter 7** we could not find associations between smoking, HLA-SE and autoantibodies other than ACPA, i.e. RF, AAPA and anti-CarP. Even though our goal was to determine when certain risk factors exerted their effected, we also explored the predictive value of AAPA and anti-CarP. Thus far conflicting findings on predictive and additional value of anti-CarP were reported, and AAPA was never studied in arthralgia-patients. With data from two arthralgia-cohorts we showed that only AAPA, but not anti-CarP, associated with development of IA independently from ACPA and RF. Further research is necessary to validate our findings.

Overall considerations from Part II

Until large population-based studies are performed, we can only speculate about the biological mechanisms behind our findings in **Chapter 6 and 7**. Since no association was found between HLA-SE and autoantibodies in the asymptomatic stage, it seems

likely that smoking, rather than HLA-SE, stimulates the initial break of tolerance to citrullinated antigens. Autoantibody-response maturation is likely stimulated by HLA-SE, which is suggested by the tendency of HLA-SE to associate with higher ACPA-levels. An increase in autoantibody levels or number of autoantibodies and isotypes occurs before symptom-development. After symptom-onset the autoantibody-response, as measured in **Chapter 6**, generally remains unchanged and does not provide a final hit towards development of IA. Other forms of autoantibody maturation than measured in **Chapter 6**, e.g. changes in cross-reactivity, affinity maturation, epitope spreading and glycosylation profile, could occur in the symptomatic phase. It is tempting to hypothesize that these forms of maturation might be involved in the final hit towards development of clinical arthritis. However, since these autoantibody characteristics associate with autoantibody levels, it seems more likely that the final hit is influenced by factors other than the autoantibody response, e.g. by other processes of the adaptive immune system or other (yet) unknown factors unrelated to the autoantibody response.

Our findings suggest that the HLA-SE might play a role in the final hit, since it significantly associated with IA-development in ACPA-positive patients in **Chapter 7**. An HLA-SE restricted T-cell response potentially stimulates the already existing ACPA-response. ACPA-IgG variable-domain glycosylation has indeed been shown to increase towards symptom-onset and significantly associates with HLA-SE.[46] While the exact mechanism remains to be elucidated, influences of HLA-SE on the ACPA-response could explain why ACPA-positive patients with HLA-SE more often develop RA than ACPA-positive patients without HLA-SE. Nevertheless, since also RA-patients without HLA-SE and/or autoantibodies present with similar clinical manifestations, other triggers remain to be elucidated.

As previously addressed in **Part I** of this thesis, a large part of RA-patients is autoantibody-negative. Apart from improving prediction in this RA-subset, knowledge on disease pathogenesis of autoantibody-negative disease needs to be enhanced. In **Chapter 7** initial analyses suggested that HLA-SE was somewhat predictive for development of ACPA-negative IA. However, this could not be replicated with the outcome RA. Since most at-risk cohorts only include autoantibody-positive patients we were not able to further explore these findings. Nevertheless, previous research suggested that HLA-SE is also involved in ACPA-negative RA, though with a smaller effect size.[47] This suggests that an effect of HLA-SE with RA-development might be found when studying a larger group of autoantibody-negative at-risk patients.

Future perspectives

Early detection and knowledge on disease pathogenesis of RA have tremendously improved over recent years, and first steps towards prevention have been taken. Nevertheless, further research is necessary to improve risk stratification and understanding of disease development.

The following points might be topic of future studies:

- A search for new clinical tests that associate with predictive imaging features, e.g. for MCP extensor peritendinitis which has shown high predictive value in CSA.
- The value of difficulties making a fist and the squeeze test may be tested in other populations, i.e. primary care, where they might prove valuable in determining presence of subclinical inflammation or risk for inflammatory disease.
- Further optimization of prediction rules, in which the value of newly discovered imaging features (e.g. juxta-articular inflammation as inter-metatarsal bursitis) or biomarkers (e.g. AAPA and/or ACPA-IgG glycosylation) need to be determined. With the prospect of preventive treatment in the future, the risk of overtreatment must be evaluated during development of new prediction rules.
- Findings on autoantibody-response maturation and timing of effects of HLA-SE and smoking as described in this thesis are ultimately confirmed in longitudinal population-based studies.
- Further research on disease pathogenesis to establish which factors are involved in the final hit towards development of RA. Factors may concern other forms of autoantibody maturation than addressed in this thesis, other processes of the adaptive immune system or even (yet) unknown factors entirely unrelated to the autoantibody response. Knowledge on timing of contributing factors may help to better target treatment in pre-clinical stages.
- Development of autoantibody-negative at-risk cohorts. While the CSA-cohort is useful in identification of predictors for autoantibody-negative RA, large cohorts for validation are lacking. Apart from identification of predictors, additional autoantibody-negative cohorts can be used to further elucidate disease processes in development of autoantibody-negative RA.

Final conclusions

In this thesis we have investigated CSA-patients and reported on the value of several clinical, imaging and immunological factors for prediction of RA. We also added to knowledge on disease pathogenesis and timing of disease processes by investigating immunological, genetic and environmental factors contributing to RA-development

in both asymptomatic and symptomatic disease phases. Although progress has been made, with the current knowledge and risk stratification it is not yet recommended to start preventive DMARD-treatment outside research settings in the absence of clinically apparent inflammatory arthritis. To prevent overtreatment, it is necessary to refrain from DMARD-treatment until adequate risk assessment is established, and clinical trials investigating the effect of preventive treatment have proven its value. Until such times, research should focus on the natural disease course of RA-development, further optimizing prediction and knowledge on disease mechanisms. In time, this could ultimately lead to prevention of RA in a high risk population, with the right treatment, at the right time.

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9

Nederlandse samenvatting

Introductie

Reumatoïde artritis (RA) is een chronische auto-immuunziekte die wordt gekenmerkt door ontstekingen van de gewrichten. Patiënten presenteren zich doorgaans met pijn, zwelling en stijfheid van de kleinere gewrichten van handen en voeten, hoewel grote gewrichten ook aangedaan kunnen zijn. Artritis kan lijden tot functionele beperkingen, en op de lange termijn ook tot schade aan het omliggende kraakbeen en bot. De ziekte kan ook systemische verschijnselen tot gevolg hebben, zoals cardiovasculaire aandoeningen, infecties en een verhoogd overlijdensrisico. Arbeidsongeschiktheid en hoge zorg- en medicatiekosten zijn gevolgen van RA op maatschappelijk niveau.

Er is nog veel onbekend over het ontstaan van RA, toch zijn er enkele risicofactoren vastgesteld. De belangrijkste omgevingsfactor is roken, de belangrijkste genetische factor is de *human leukocyte antigen-shared epitope* (HLA-SE). Roken en HLA-SE zijn voornamelijk geassocieerd met ontwikkeling van autoantistof-positieve ziekte. De ziekte kan grofweg worden ingedeeld in autoantistof-positieve en autoantistof-negatieve RA, bij ongeveer 50% van de vroege RA-patiënten zijn autoantistoffen aanwezig. De autoantistoffen die algemeen worden erkend en in de klinische praktijk het meest worden gebruikt zijn *reumafactor* (RF) en *anti-citrullinated protein antibodies* (ACPA). Gewrichtsschade en ernstige lange termijn uitkomsten worden voornamelijk geassocieerd met ACPA-positieve RA. Vrouwen worden vaker door de ziekte getroffen, ongeveer 75% van de patiënten is vrouw.

De behandeling van RA is in de laatste decennia sterk verbeterd. Hoewel het in het verleden niet uitzonderlijk was dat de ziekte tot ernstige gewrichtsschade leidde, wordt dit tegenwoordig vaak voorkomen. Een belangrijke bijdrage aan deze verbetering is de vroege herkenning en behandeling van de ziekte. Het wordt aanbevolen om binnen 12 weken na presentatie met gewrichtszwelling de behandeling met reuma-medicatie (*disease modifying anti-rheumatic drugs*, DMARDs) te starten, hiermee wordt volledige maturatie van de ziekte en onomkeerbare schade voorkomen. Het is nog onbekend of eerdere behandeling het ontstaan van gewrichtszwelling helemaal zou kunnen voorkomen. Om preventieve behandeling mogelijk te maken, is het noodzakelijk de RA-patiënten te herkennen nog voor gewrichtszwelling ontstaat, en te weten welke ziekteprocessen zich tijdens het ontstaan van de ziekte afspelen.

In dit proefschrift kwamen twee hoofddoelen aan de orde. **Deel I** van dit proefschrift richtte zich op het verbeteren van de voorspelling en vroege detectie van RA. Dit hebben wij onderzocht in een groot cohort van patiënten met klinisch verdachte artralgie (*clinically suspect arthralgia*, CSA); bij deze patiënten is nog geen sprake van

gewrichtszwelling, maar door de klinische presentatie vermoedt de reumatoloog dat deze patiënten in de toekomst RA zullen ontwikkelen. In **Deel II** was het doel de kennis over ziekteprocessen die ten grondslag liggen aan de ontwikkeling van RA verder uit te breiden. Wij onderzochten welke ziekteprocessen betrokken zijn bij de verschillende fasen van ontwikkeling van RA.

Deel I – Voorspelling en vroege detectie van reumatoïde artritis

De waarde van beeldvorming bij de voorspelling van RA is vaak onderzocht. Subklinische inflammatie kan worden gezien zelfs vóór het optreden van klinisch detecteerbare artritis en het is aangetoond dat aanwezigheid hiervan ziekteprogressie voorspelt. Beeldvormende technieken zoals echografie en MRI zijn echter kostbaar, tijdrovend en niet altijd beschikbaar. Daarbij is er mogelijk een associatie tussen subklinische inflammatie en klinische verschijnselen die worden beschouwd als risicofactoren voor het ontwikkelen van RA. In **Hoofdstuk 2** onderzochten we daarom één van deze klinische verschijnselen; problemen bij het maken van een vuist, een van de factoren die ook in de EULAR-definitie van klinisch verdachte artralgie is opgenomen. Hoewel vuistproblemen worden beschouwd als een risicofactor voor het ontwikkelen van RA bij patiënten met CSA, de voorspellende waarde en onderliggende oorzaak waren onduidelijk. Problemen bij het maken van een vuist werd op twee manieren beoordeeld: 1) het vermogen om de vuist volledig te sluiten, waarbij alle vingertoppen de handpalm raken, werd geëvalueerd door visuele inspectie, 2) de vuistkracht werd bepaald door de patiënt in de vingers van de beoordelaar te laten knijpen. Onvolledige vuistsluiting en een verminderde vuistkracht waren beide onafhankelijk geassocieerd met ontwikkeling van klinisch aantoonbare inflammatoire artritis (IA), hierbij had onvolledige vuistsluiting een hogere voorspellende waarde en betere betrouwbaarheid. Vuistproblemen associeerden significant met flexor tenosynovitis; onvolledige vuistsluiting associeerde voornamelijk met flexor tenosynovitis van metacarpofalangeale (MCP) gewrichten, terwijl verminderde vuistkracht sterker gerelateerd was aan flexor tenosynovitis van de pols. Deze resultaten laten zien dat problemen bij het maken van een vuist, en voornamelijk vuistsluiting, gemakkelijk te beoordelen zijn in de klinische praktijk en informatie kunnen geven over zowel het risico voor ziekteprogressie als onderliggende flexor tenosynovitis.

Naast vuistproblemen werd de waarde van een andere eenvoudige klinische test in CSA onderzocht. Historisch gezien werd de *squeeze* test, d.w.z. compressie rondom de knokkels van MCP en metatarsofalangeale (MTP) gewrichten, gebruikt om

aanwezigheid van synovitis te beoordelen. Bij vroege artritis is inderdaad aangetoond dat een positieve squeeze test geassocieerd was met de aanwezigheid van synovitis in MCP- en MTP-gewrichten, en zelfs met lokale MRI-gedetecteerde inflammatie. In de fase van CSA wordt de squeeze test beschouwd als een risicofactor voor de ontwikkeling van RA, aangezien deze test ook is opgenomen in de EULAR-definitie van klinisch verdachte artralgie. De hypothese ontstond dat een positieve squeeze-test bij CSA, in afwezigheid van klinische artritis, geassocieerd zou kunnen zijn met aanwezigheid van subklinische inflammatie; dit werd onderzocht in **Hoofdstuk 3**. Er werd aangetoond dat >50% van de CSA-patiënten een positieve squeeze test had in MCP- of MTP-gewrichten, en dat een positieve test onafhankelijk geassocieerd was met lokale subklinische synovitis met een odds ratio (OR) van 2. De sensitiviteit van de test was slechts 44%, wat aangeeft dat subklinische synovitis ook vaak wordt gemist. Bovendien was een positieve squeeze test bij CSA niet onafhankelijk geassocieerd met progressie naar IA. Ondanks het ontbreken van voorspellende waarde, is de squeeze test een eenvoudige en snelle test die kan worden gebruikt om een eerste indruk te krijgen van de aanwezigheid van subklinische synovitis.

Wanneer subklinische inflammatie in personen met een verhoogd risico op RA wordt aangetoond met beeldvorming, wordt behandeling soms overwogen, zelfs als er geen klinische artritis aanwezig is. Studies hebben aangetoond dat een toenemend aantal reumatologen een DMARD-behandeling overweegt of initieert bij patiënten met autoantistof-positieve artralgie, hun keuzes worden gestuurd door bevindingen met echografie en de aanwezigheid van subklinische inflammatie. Subklinische inflammatie kan inderdaad voorafgaan aan de ontwikkeling van IA, maar subklinische inflammatie en symptomen verdwijnen ook vaak spontaan. In **Hoofdstuk 4** hebben we daarom de waarde van subklinische synovitis bepaald, zowel de waarde van subklinische synovitis als startpunt voor DMARD-behandeling maar ook de potentie voor overbehandeling. We bestudeerden drie artralgiecohorten waarin de aanwezigheid van subklinische synovitis bij aanvang werd bepaald door echografie of MRI. Alle patiënten werden gedurende één jaar gevolgd voor de ontwikkeling van IA, waarbij DMARD-behandeling (inclusief corticosteroïden) niet was toegestaan. Bij ACPA-positieve patiënten met subklinische synovitis ontwikkelde 50-68% van de patiënten geen IA, bij ACPA-negatieve patiënten was dit 66-89%. Zelfs bij patiënten met aanvullend ≥6 punten op de 2010 EULAR-classificatiecriteria voor RA, bleef het aantal dat geen IA ontwikkelde aanzienlijk (≥37%). De resultaten bleven ook vergelijkbaar wanneer strengere definities van subklinische synovitis werden gebruikt. Deze bevindingen geven aan dat DMARD-behandeling bij artralgiepatiënten met subklinische synovitis zou leiden tot aanzienlijke overbehandeling.

Subklinische inflammatie zoals gemeten in **Hoofdstuk 2, 3 en 4**, is zelfs

gecombineerd met ACPA-status onvoldoende voor goede identificatie van patiënten die in de toekomst IA ontwikkelen. Andere kenmerken die met beeldvorming kunnen worden weergegeven verhogen mogelijk de prognostische waarde van subklinische inflammatie. Boterosies zijn een kenmerk van RA, en er zijn zelfs RA-specifieke MRI-gedetecteerde erosies vastgesteld; deze erosies waren aanwezig bij patiënten met vroege RA, maar niet bij patiënten met andere artritiden. MRI heeft een hoge sensitiviteit voor detectie van erosies; zelfs bij personen zonder symptomen en bij patiënten met CSA zijn kleine erosies detecteerbaar. In **Hoofdstuk 5** hebben we MRI-gedetecteerde erosies in de fase van CSA onderzocht. We onderzochten de voorspellende waarde van MRI-gedetecteerde erosies voor de ontwikkeling van IA, en evalueerden of de prognostische waarde van MRI-gedetecteerde subklinische inflammatie kon worden verbeterd door evaluatie van MRI-gedetecteerde erosies. Een MRI-erosie, gedefinieerd als erosies die aanwezig waren bij <5% van de symptoomvrije personen in hetzelfde bot en in dezelfde leeftijdscategorie, was aanwezig bij 20% van de CSA-patiënten. Aanwezigheid van deze erosies was niet geassocieerd met IA-ontwikkeling. Erosies die eerder werden gerapporteerd als specifiek voor RA (graad ≥2 erosies, erosies in MTP5 en erosies in MTP1 bij personen <40 jaar) werden zelden gezien bij CSA-patiënten, en hun aanwezigheid was niet geassocieerd met IA-ontwikkeling. Wanneer MRI-gedetecteerde erosies werden geëvalueerd in toevoeging tot MRI-gedetecteerde subklinische inflammatie, verbeterde de *area under the curve* (AUC) niet en nam de prognostische waarde af. Dit bleek ook uit de *net reclassification index* (NRI) van -5,8; het aanvullend evalueren van MRI-gedetecteerde erosies resulteerde in een groot aantal fout-positieve voorspellingen. Aangezien erosies vooral vroeg optreden bij ACPA-positieve ziekte, werden MRI-erosies ook afzonderlijk geëvalueerd in ACPA-positieve en ACPA-negatieve CSA-patiënten. In geen van beide subsets waren MRI-gedetecteerde erosies voorspellend voor de ontwikkeling van IA. De mediane erosiescore bij ACPA-positieve patiënten was echter significant hoger dan bij ACPA-negatieve patiënten. Dit verschil werd alleen gezien bij patiënten met subklinische ontsteking; ACPA-positieve patiënten zonder subklinische ontsteking hadden geen hogere erosiescore dan ACPA-negatieve patiënten zonder subklinische ontsteking.

Deel II – Pathogenese van reumatoïde artritis

Juiste identificatie van patiënten met een verhoogd risico op het ontwikkelen van RA kan worden verbeterd door inzicht te krijgen in de pathogenese van de ziekte. Kennis over ziekteprocessen en timing van factoren die van invloed zijn bij het begin en tijdens progressie van de ziekte kunnen helpen om de juiste behandeling in preklinische stadia te bepalen, met als uiteindelijk doel het voorkomen van RA.

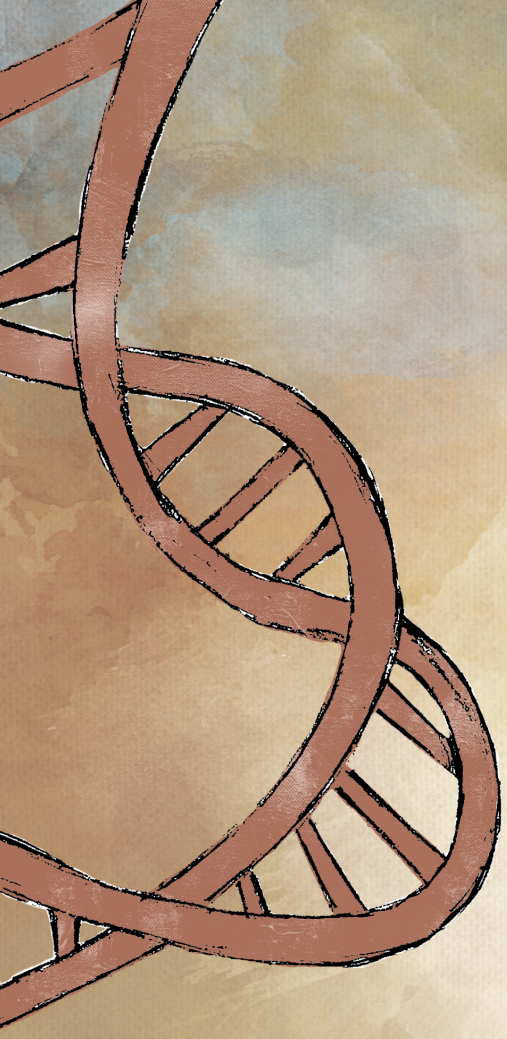
Ontwikkeling van autoantistoffen en maturatie van de autoantistof-respons, gedefinieerd als een toename van het aantal autoantistoffen en autoantistof-levels, gaan vooraf aan de ontwikkeling van RA. Het was niet bekend of maturatie van autoantistoffen nog plaats vindt in de fase van CSA, of dat de respons al volledig is uitgerijpt als symptomen ontstaan. In **Hoofdstuk 6** evalueerden we daarom de aanwezigheid en levels van autoantistoffen in CSA op twee momenten. Bij patiënten die IA ontwikkelden werd bloed afgenomen op baseline en op het moment van IA-ontwikkeling, bij patiënten die geen IA ontwikkelden werd bloed afgenomen op baseline en na twee jaar. Als maturatie van de autoantistof-respons een rol speelt bij ziekteprogressie, werd verwacht dat maturatie alleen aanwezig zou zijn bij CSA-patiënten die IA ontwikkelden. We analyseerden drie autoantistoffen (ACPA, a*nti-carbamylated protein antibodies* (anti-CarP) en *anti-acetylated protein antibodies* (AAPA)) in drie verschillende isotypen (IgM, IgG en IgA). Patiënten zonder autoantistoffen op baseline ontwikkelden zelden autoantistoffen tijdens follow-up. In patiënten met ≥1 autoantistof (van de negen) op baseline was het mediane aantal autoantistoffen 1, en een toename van het aantal autoantistoffen tijdens follow-up kwam niet vaak voor. Autoantistof-levels veranderden niet significant tijdens follow-up. De bevindingen waren vergelijkbaar tussen patiënten die IA ontwikkelden en patiënten die geen IA ontwikkelden. We concludeerden daarom dat maturatie van autoantistoffen niet doorslaggevend was voor de ontwikkeling van klinisch aantoonbare artritis. Echter, wanneer de uitkomst RA werd gebruikt, d.w.z. dat op het moment van IA-ontwikkeling ook werd voldaan aan de ACR/EULAR-criteria van 1987 en/of 2010, was in patiënten die geen RA ontwikkelden tijdens follow-up een afname te zien in het mediane aantal autoantistoffen. Mogelijk ontbraken bij deze patiënten andere factoren die betrokken zijn bij het in stand houden van de autoantistof-respons.

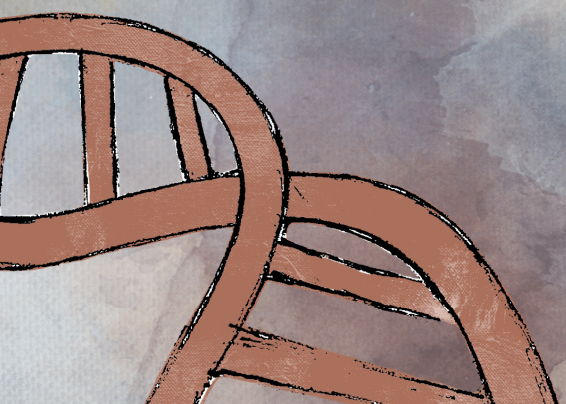
In **Hoofdstuk 7** hebben we ons gericht op de rol en timing van twee prominente risicofactoren voor de ontwikkeling van RA; genetische factor HLA-SE en omgevingsfactor roken. Hun associatie met RA wordt algemeen erkend, hoewel het niet bekend is in welk ziektestadium ze hun effect uitoefenen. Om een mogelijke rol van HLA-SE en roken bij de ontwikkeling van autoantistoffen te onderzoeken, hebben we literatuur bestudeerd over associaties van HLA-SE en roken met de aanwezigheid van ACPA in asymptomatische populaties. Meta-analyses lieten zien dat roken, maar niet HLA-SE, geassocieerd was met ACPA-positiviteit bij asymptomatische individuen. Op het moment dat symptomen aanwezig zijn (aanvang CSA), zijn zowel HLA-SE als roken geassocieerd met de aanwezigheid van ACPA. Hoewel eerdere studies interacties hebben gevonden tussen genetische en omgevingsfactoren voor de ontwikkeling van ACPA, was in CSA de associatie van roken met ACPA niet afhankelijk van de aanwezigheid van HLA-SE, en werd er geen significante interactie tussen HLA-SE en roken gevonden. Daarnaast konden eerdere bevindingen in RA, dat roken

niet geassocieerd was met ACPA maar met RF of autoantistoffen in het algemeen, niet worden gerepliceerd in meta-analyse van asymptomatische populaties of in CSA-patiënten. Tijdens follow-up was HLA-SE geassocieerd met progressie naar IA in de totale CSA-populatie, en meta-analyse in drie artralgie-cohorten toonde dezelfde associatie in de ACPA-positieve subset. Roken was niet geassocieerd met IA-ontwikkeling, niet in de totale CSA-populatie, na ACPA-stratificatie of in meta-analyse met andere cohorten. Samen impliceren de resultaten van deze studie dat roken betrokken is bij de ontwikkeling van autoantistoffen en mogelijk bij de ontwikkeling van symptomen, maar niet bij verdere progressie naar IA. HLA-SE is niet betrokken bij de ontwikkeling van autoantistoffen, speelt mogelijk een rol bij de maturatie van autoantistoffen en de ontwikkeling van symptomen, en is geassocieerd met IA-ontwikkeling.

Conclusies

In dit proefschrift hebben we CSA-patiënten onderzocht en gerapporteerd over de waarde van klinische tests, beeldvorming en immunologische factoren voor het voorspellen van RA. Ook hebben we de kennis over de pathogenese en timing van ziekteprocessen vergroot door immunologische, genetische en omgevingsfactoren te onderzoeken die bijdragen aan de ontwikkeling van RA in zowel asymptomatische als symptomatische ziektefasen. Hoewel er vooruitgang is geboekt, wordt met de huidige kennis nog niet aanbevolen om preventieve DMARD-behandeling buiten onderzoek setting te starten zolang klinisch aantoonbare inflammatoire artritis afwezig is. Om overbehandeling te voorkomen, is het noodzakelijk om te wachten met DMARD-behandeling tot we nog beter in staat zijn te voorspellen wie in de toekomst RA zal ontwikkelen en klinische onderzoeken de waarde van preventieve behandeling hebben aangetoond. Tot die tijd moet het onderzoek zich richten op het natuurlijke ziekteverloop van RA-ontwikkeling, waarbij het voorspellen van RA en kennis over ziektemechanismen verder worden geoptimaliseerd. Op termijn kan dit uiteindelijk leiden tot preventie van RA, door het geven van de juiste behandeling op het juiste moment, in personen met een hoog risico op RA-ontwikkeling.





Appendices

Curriculum Vitae
List of publications
Dankwoord

Curriculum Vitae

Fenne Wouters is geboren op 26 juli 1992 in Tiel. Zij groeide op in Culemborg en behaalde in 2010 haar VWO diploma bij O.R.S Lek en Linge.

Zij vervolgde haar opleiding bij de Hogeschool Utrecht, waar zij de verkorte opleiding Fysiotherapie volgde. In 2013 studeerde zij af, waarna zij twee jaar heeft gewerkt als waarnemend fysiotherapeut bij Fysiotherapeuten Maatschap Woerden, zowel in de particuliere praktijken van de Maatschap, als in de kliniek van het Zuwe Hofpoort Ziekenhuis.

In 2014 startte zij met de premaster Bewegingswetenschappen aan de Vrije Universiteit van Amsterdam, die zij in 2015 vervolgde met de research master Human Movement Sciences. In haar laatste studiejaar verrichtte zij celbiologisch onderzoek naar de ziekte Fibrodysplasia Ossificans Progressiva. In 2017 studeerde zij cum laude af.

In mei 2018 is zij begonnen aan haar promotieonderzoek op de afdeling Reumatologie van het Leids Universitair Medisch Centrum, onder begeleiding van prof. dr. A.H.M. van der Helm-van Mil en dr. E. Niemantsverdriet.

Momenteel is zij werkzaam als onderzoeker op de afdeling Ouderengeneeskunde van het Amsterdam Universitair Medisch Centrum. Hier werkt zij binnen het programma Leren van Data aan het verbeteren van (her)gebruik van zorggegevens die door specialisten ouderengeneeskunde in verpleeghuizen worden vastgelegd.

List of publications

Wouters F, van Loon AM, Rutten JJS, Smalbrugge M, Hertogh C, Joling KJ. Risk of Death in Nursing Home Residents After COVID-19 Vaccination. J Am Med Dir Assoc. 2022.

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