Psychological and biological features influencing the risk for rheumatoid arthritis

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RHEUMATOID ARTHRITIS

Marian van Beers-Tas

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Marian H. van Beers-Tas

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PSYCHOLOGICAL AND BIOLOGICAL FEATURES INFLUENCING THE RISK FOR RHEUMATOID ARTHRITIS

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CHAPTER 1

General introduction

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is characterized by joint pain and synovial inflammation, and is associated with structural damage and premature mortality[1-3]. It is usually diagnosed shortly after the appearance of clinically apparent inflammatory arthritis (IA). The main features of RA are summarized in the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria as shown in **Table 1**[4]. RA is present in 0.5-1% of Caucasians, becomes manifest on average around the age of 55 years, and is more prevalent in women compared to men[5]. Due to its relatively high prevalence and negative impact on functional ability, even before the diagnosis, it poses an enormous burden to patients, their families and the health care system[6]. In was estimated to be on the 22th place regarding disability adjusted life years (DALYs) in the Netherlands in 2015. DALYs represent the sum of years a person lives shorter due to the disease (i.e. lost life years) and the loss of quality of life (i.e. life year equivalents)[7]. Also, health care costs are high (568 million in 2011[8]). Therefore, a constant search for new ways to predict and possibly even to prevent RA is warranted[9]. At present screening for RA risk is still experimental, since there is no validated screening tool and no proven therapy that can prevent the disease.

Table 1. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis (RA)

Target population (who should be tested?): patients who		
	1) have at least 1 joint with definite clinical synovitis (swelling)	
	2) with the synovitis not better explained by another disease	
Classification criteria for RA		Score
	Score-based algorithm: add score of categories A-D	
	A score of ≥6/10 is needed for classification of a patient as having definite RA	
A	*Joint involvement**	
	1 large joint**	0
	2-10 large joints	1
	1-3 small joints (with or without involvement of large joints)***	2
	4-10 small joints (with or without involvement of large joints)	3
	>10 joints (at least 1 small joint)	5
B	*Serology (at least one test result is needed for classification)* ****	
	Negative RF *and* negative ACPA	0
	Low-positive RF *or* low-positive ACPA	2
	High-positive RF *or* high-positive ACPA	3
C	*Acute-phase reactants (at least one test result is needed for classification)*	
	Normal CRP *and* normal ESR	0
	Abnormal CRP *or* abnormal ESR	1

table continues

Classification criteria for RA		Score
D	*Duration of symptoms******	
	<6 weeks	0
	≥6 weeks	1
*	Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal joints, first carpometacarpal joints, and first metatarsophalangeal joints are excluded from assessment	
**	Large joints: shoulders, elbows, hips, knees and ankles	
***	Small joints: metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints and wrists	
****	Low-positive refers to IU valueas that are higher than the upper limit of normal (ULN), but ≤3 times the ULN. High-positive refers to IU values that are >3 times the ULN for the laboratory and assay	
*****	Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g. pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status	
Abb	Abbreviations: RA=rheumatoid arthritis, RF=rheumatoid factor, ACPA=anti-citrullinated protein antibodies, CRP=C-reactive protein, ESR=erythrocyte sedimentation rate	

THE AT-RISK PHASE OF RHEUMATOID ARTHRITIS

RA is a multifactorially determined disease with a very heterogeneous phenotype. It is thought that genetic susceptibility, immune dysregulation and environmental factors all play a role in its pathogenesis (Figure 1)[10 11]. It is now known that the majority of individuals go through a phase of autoimmunity accompanied by subclinical inflammation, followed by a symptomatic phase which may last from months to several years[12 13]. In order to develop systemic auto-immunity, it is believed that a "second hit" such as infections or immune-specific inflammation at extra-articular sites such as the mouth, lungs or gut is necessary to trigger auto-antibody formation, however, the timing of these events is not yet clear[14-18]. During the ensuing symptomatic phase, markers of autoimmunity and inflammation increase before the onset of clinical arthritis. In the at-risk phase in which individuals have symptoms without arthritis the joint pain or discomfort is named arthralgia. The recently coined term "clinically suspect arthralgia (CSA)" indicates individuals of whom the rheumatologist suspects they will progress to arthritis based on the symptom pattern[19]. In this symptomatic phase auto-antibodies (such as rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA)) may or may not be detectable. Depending on the type of investigated cohort the percentage of individuals progressing to arthritis may vary widely. For instance, in the Reade prospective cohort of seropositive arthralgia patients, 35% developed arthritis within a 5-year period (of whom 90% fulfilled the 2010 ACR/EULAR classification criteria for RA)[20]. However, the percentage in the Leiden prospective cohort of CSA patients was lower (20%)[21], and was also estimated lower in persons with multiple first-degree relatives with RA (3.9% in 5 years)[22 23].

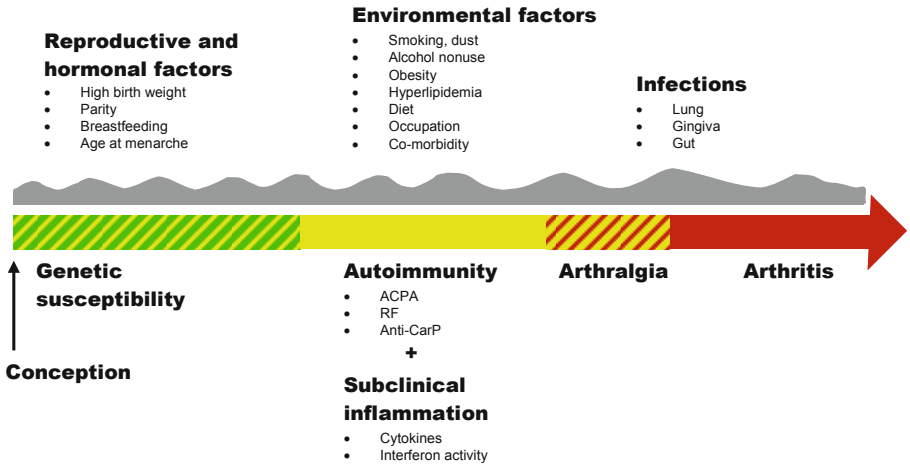

Figure 1. The evolution of RA from health to disease. ACPA, anti–citrullinated protein antibody; RF, rheumatoid factor; anti-CarP, anti-carbamylated protein antibodies.

PREDICTING DEVELOPMENT OF RHEUMATOID ARTHRITIS

No validated screening tools for RA have been described. However, multiple attempts have been made to unravel the relation of risk factors with the development of RA. This was performed in selected high risk populations, such as individuals with arthralgia and auto-antibodies in the blood (RF and/or ACPA), CSA patients or first degree relatives of RA patients. Future research in other study cohorts such as the general population may receive more attention in the Netherlands as a large registry is available containing data with long time follow-up of a large part of the Dutch inhabitants. This database (described further) contains information on all symptoms and diseases for which an individual contacts the general practitioner. With new algorithms to enhance certainty of diagnoses this may aid in future earlier detection of patients at risk of developing RA.

As mentioned in the previous paragraph research has focused on risk factors such as genetic markers, auto-antibodies, symptom patterns and environmental factors. Heritability is around 65% based on twin studies, however, even though >100 risk loci have been identified[24-26], the known RA genetic risk factors taken together only explain ~16% of the total susceptibility (heritable and environmental)[27]. Most of these risk alleles on their own convey only a small risk of RA, but mainly multiple alleles of the HLA-DRB1 complex called the shared epitope have been associated with a 5.4-fold higher risk of RA, which increased to 21-fold in smokers[18]. This illustrates the role of smoking as the major environmental risk factor [28 29]. On the contrary, there are also protective genetic factors, such as the HLA-DRB1-13 alleles[30]. The best studied risk factors are the auto-antibodies RF and ACPA. Approximately two-thirds of RA patients test positive for RF and/or ACPA at diagnosis, with a high specificity, underscoring their importance in this disease. After an enzyme-linked immunosorbent assay (ELISA) test for ACPA became available around 2000, two blood

donor cohort studies were the first to investigate RF and ACPA in individuals before the RA diagnosis. These showed that the presence of auto-antibodies was more prevalent in later RA patients as compared to matched controls. Rantapää-Dahlqvist et al showed that for RF this was 19.3% compared to 6.0%, and for ACPA this was 33.7% compared to 1.8% in the 10 years before diagnosis[31]. Similar results were found by Nielen et al at a mean of 5 years before RA development; 27.8% versus 1.1% for RF and 40.4% versus 0.6% for ACPA[23]. Other autoantibody tests and immune parameters have added varying predictive value [32], such as anti-carbamylated protein (CarP) antibodies[33], the type 1 interferon signature[34], and various B-cell markers[35 36].

Finally, through assembling cohorts of patients with recently diagnosed RA or those at-risk of developing RA one can investigate their symptoms. Symptoms such as joint pain, swelling and morning stiffness represent key elements in the diagnosis of RA. Clinicians have tried to use these and other reported symptoms to characterize those at risk of RA before they fulfil classification criteria[37]. A EULAR taskforce recently outlined symptoms and signs that were deemed most relevant in identifying subjects with CSA, a category of patients at risk of developing RA[19]. This undertaking was from the perspective of the rheumatologist, who had to label arthralgia patients as CSA or not. Qualitative research in individuals at risk of RA provided a different starting point to evaluate symptoms, using the experience of the affected persons to understand the range of their symptomatology. With this approach focus group interviews were performed in seropositive arthralgia patients[37-39]. Both approaches can be used for prediction purposes and first results have been published[40 41].

Besides attempts to use individual risk factors or biomarkers for prediction, several authors have also tried to combine them. Both genetic[42-46] and clinical[20 44 47-49] prediction models, or combinations of these[50] have been made. So far these are not accurate enough for use in individual patient care.

AT-RISK POPULATIONS

Both the general population and high risk populations have been used to investigate the at-risk phase of RA. The general population was used for instance in the population-based incidence cohort of Minnesota[51], the Nurses' Health study from the US[52], the Women's health study from the US[53] and the Malmö Diet and Cancer Study from Sweden[54]. The high-risk population of Pima Indians of Arizona was the first cohort to be followed longitudinally[55]. Increased titers of RF were found in those that later developed RA as compared to those who did not. Not long thereafter another high-risk population of families with more than one member having RA confirmed this conclusion[22 56]. These were the first studies in which the at-risk phase, then called pre-RA, was investigated. Within the same time period Aho et al discovered that antikeratin and antiperinuclear factor antibodies (both later identified as ACPA) were present in RF positive RA patients before they developed the disease[57]. Much later blood bank data of RA patients before they were diagnosed appeared[23 31 58]. It was not until 2004 that individuals at higher risk of developing RA based on auto-antibodies were

included in prospective cohorts. The Reade seropositive arthralgia cohort was the first (described below), and was set up to determine the effect of dexamethasone on arthritis development[59 60]. After 2005 the publication rate on the at-risk phase has increased rapidly[9]. Some other contributors to data from prospective cohorts are the AMC Amsterdam at-risk cohort[36 47], the Studies of the Etiology of RA (SERA) group in the US[61], the Canadian North American Native population[62] and the CSA cohort of Leiden[63]. Ongoing follow-up in these cohorts and among others the UK (seropositive and seronegative CSA patients), Sweden (ACPA-positive patients), France (seropositive patients) and Switzerland (first-degree relatives of patients with RA) open the doors for unravelling the processes before clinical RA. Also, data from other settings than the secondary health care system might add future information, such as primary care databases. As the incidence of inflammatory arthritis in primary care practices in the Netherlands is very low (400 patients with joint symptoms per year in an average practice, of which only 6 received an inflammatory arthritis diagnosis[64]) it is important to note that large databases are needed to be able to find risk predictors.

AT-RISK POPULATIONS USED FOR THIS THESIS

Prospective cohort from Reade of seropositive arthralgia patients
This cohort was set up in 2004 in Amsterdam. It includes individuals referred with arthralgia that are positive for RF and/or ACPA. It was formed to identify clinical and serological predictors for the development of arthritis. In the first years patients were asked to participate in a randomised controlled trial investigating the role of dexamethasone in preventing arthritis. This medication did not prove to be effective[59], but the cohort continued and is still ongoing, now including over 600 individuals. Demographic, clinical and laboratory measurements are performed every year until a complete follow-up of 5 years or arthritis development. Arthritis development (35%), if present, is confirmed by a senior rheumatologist without knowledge of the serostatus. This cohort is used in chapters 4, and 7-10 of this thesis.

International convenience sample
Although over 600 individuals in one cohort at risk of RA is a fair amount, to make data more generalizable and also to be able to obtain data on the development of a symptom questionnaire faster (chapter 5), we combined data from 5 European centers, including the one from Reade described above: From Birmingham (UK) patients were included with CSA that could be seropositive or negative; from Stockholm (Sweden) patients with musculoskeletal symptoms testing positive for ACPA and referred by primary care physicians (or other specialists) to the rheumatologist; from Vienna (Austria) seropositive and seronegative arthralgia patients from the outpatient clinic or from public advertising; and finally, from Geneva (Switzerland) individuals were selected who are a first degree relative of a RA patient.

Nivel Primary Care Database (Nivel-PCD)
For chapter 6 data was used from Nivel-PCD[65]. Nivel-PCD collects data from routine electronic health records from a representative sample of approximately 500 general

practices in the Netherlands (with a total of more than 1.5 million registered patients). This includes information about consultations, morbidity, prescriptions and diagnostic tests. Diagnoses are recorded using the International Classification of Primary Care (ICPC-1) coding system[66].

THESIS OUTLINE

The aim of this thesis was to further study the at-risk phase of RA development, in a broad spectrum of at-risk populations, with a focus on symptomatology and markers (serological as well as imaging). At the end of this general introduction, in short, a summary is made of "what is known" and "what is new".

The thesis contains three parts. In **Part I,** two systematic reviews cover risk factors, screening for and prevention of RA. **Chapter 2** highlights current evidence on risk factors for RA and the prediction rules that combine these risk factors. Also, it concerns the question whether RA can be screened for, and what the implications of such possibilities are. In **Chapter 3**, the whole spectrum from the at-risk phase of RA, through undifferentiated arthritis (UA) and early RA to established RA is reviewed, including efforts to prevent RA from occurring (primary prevention) or from progressing from UA to RA (secondary prevention).

In **Part II,** it is described why symptoms such as joint pain, swelling and morning stiffness represent not only key elements for the diagnosis of RA, but can also be helpful in the at-risk phase of RA. In addition, extra-articular and systemic symptoms are investigated. To this end three populations described above were used: first seropositive arthralgia patients from the Reade cohort, second an international convenience sample, and third a large primary care database. In **Chapter 4**, the associations between depressive mood, daily stressors, avoidance coping or social support on one hand, and the development of arthritis or related clinical parameters on the other are investigated. **Chapter 5** evaluates the use of the Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire derived from focus group interviews in 15 seropositive arthralgia patients and 11 early RA patients with whom initial symptoms prior to the diagnosis of RA were explored. In **Chapter 6**, a case-control study evaluated the timing and number of visits to the GP for musculoskeletal symptoms, infections and rheumatoid arthritis-related comorbidities before the diagnosis of inflammatory arthritis.

In **Part III,** a wide range of markers for the development of RA was investigated. **Chapter 7** describes the predictive ability of serum 14-3-3ŋ (eta) which is a biomarker involved in the upregulation of inflammatory and joint damage factors. In **Chapter 8**, we studied whether RF and ACPA levels over time are just as predictive for RA as measuring the levels at just one time point as is usually done at baseline in most cohort studies. **Chapter 9** describes the validation of a new marker based on the presence of dominant b cell receptor clones in peripheral blood in individuals at risk for rheumatoid arthritis. In **Chapter 10**, with joint ultrasonography the value of synovial thickening and Power Doppler signal in a standard set of joints was investigated.

Finally, **Chapters 11 and 12** provide a summary and discussion of the study results presented in this thesis.

SUMMARY

What is known:

- Rheumatoid arthritis (RA) is a multifactorially determined disease in which genetic susceptibility, immune dysregulation and environmental factors all play a role
- Individuals at risk of developing RA usually go through a phase of autoimmunity accompanied by subclinical inflammation, followed by a symptomatic phase
- Several risk factors for developing RA are known, but at present no validated screening tool and no proven therapy that can prevent the disease are available

What is new:

- Focus was put on symptoms in the at-risk phase of RA: which symptoms are important, how can they be measured and are they predictive for RA development?
- New knowledge will be presented on: the predictive capacity of serological markers (14-3-3η, longitudinal autoantibody levels of RF and ACPA, and B-cell receptor clones) and imaging (ultrasonography) for the development of RA

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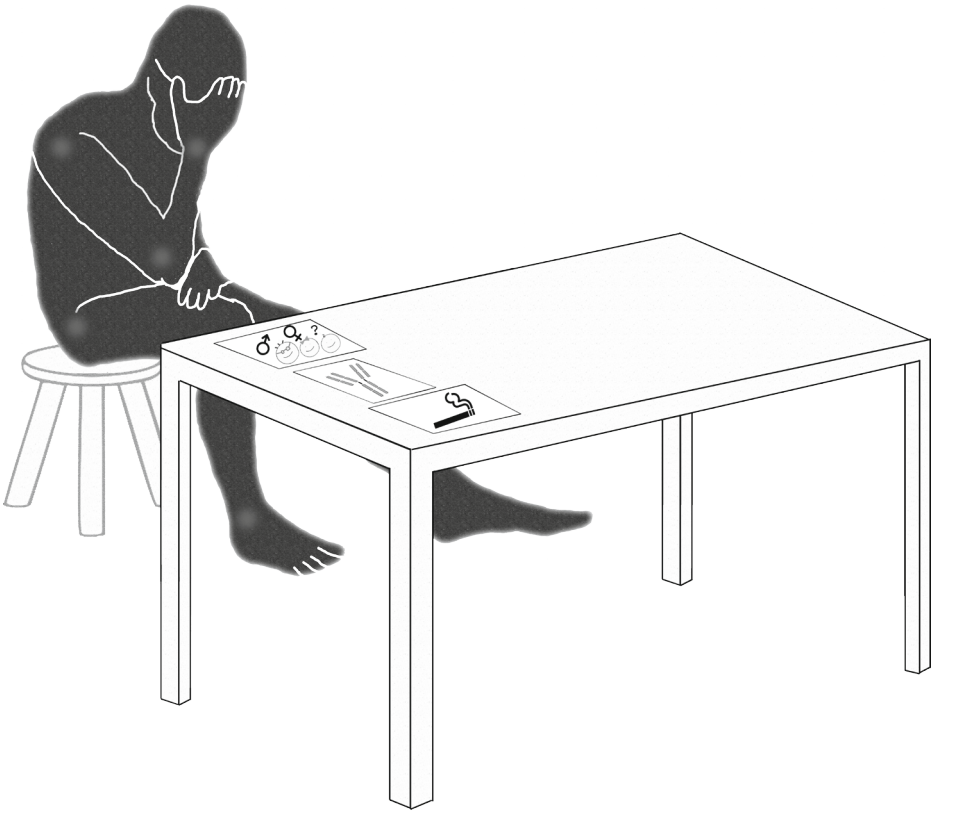
PART I

Reviewing the at-risk phase of rheumatoid arthritis



CHAPTER

2

Prediction of future rheumatoid arthritis

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KEYPOINTS

- Risk factors for rheumatoid arthritis (RA) include family history, birth weight, smoking, silica, alcohol nonuse, obesity, diabetes mellitus, autoantibodies, and genetic variants.
- Symptoms, antibodies, and inflammatory biomarkers can be useful in late at-risk stages, and genetic scores plus environmental factors more useful in early at-risk stages.
- Prediction models of RA can help to select candidates for intervention studies.
- The best target populations for screening are relatives of patients with RA and (seropositive) patients with arthralgia. However, only a minority of persons at risk can thus be recognized.
- Screening for RA risk is still experimental, because there is no validated screening tool and no proven therapy to prevent disease

INTRODUCTION

Rheumatoid arthritis (RA) on average becomes clinically manifest around the age of 55 years. During the healthy part of life, the risk of future RA is determined by genetic, reproductive and environmental factors (Fig. 1, green bar). Over time, people at risk for RA may pass through a phase of autoimmunity, accompanied by subclinical inflammation,[1] followed by a symptomatic phase, which may last a few months to several years. In the symptomatic phase, markers of autoimmunity and inflammation increase before the onset of clinical arthritis.[2] Therefore, prediction can be based on different characteristics in the asymptomatic phase and in the symptomatic phase.

The expectation that intervening in the preclinical phase of RA could be beneficial is based on the success of treatment of RA within 1 to 2 years after onset of clinical disease.[3,4] The new criteria for RA from 2010 with a focus on early signs such as involvement of even only a few small joints together with serology and acute phase reactants facilitate treatment in the earliest clinical phase,[5,6] and the further characterization of the preclinical phase offers new opportunities for intervention studies even before clinically apparent arthritis occurs. Because RA is the most prevalent inflammatory rheumatic disease, with a high burden for the patient and society, it seems the ideal candidate rheumatic disease for screening and intervention programs. However, a lot of steps need to be taken before such programs can be offered to persons at risk.

This article summarizes the present knowledge on risk factors for RA, including genetic, reproductive, and hormonal factors; environmental exposures; biomarkers; personal characteristics and symptoms; and how these can be combined in risk models attempting to increase the accuracy of the prediction of RA. Genetic risk and gene-environmental interactions are dealt with elsewhere in this issue and are only mentioned here in relation to their roles in prediction models. Risk scores from such models require further validation, but could be used to select candidates for intervention studies.

METHODS

We searched the PubMed database on January 29, 2014, for the terms risk, prediction, and development in relation to RA. After excluding articles not directly related to prediction of RA, such as studies on prevalence, diagnosis, treatment, outcome, or comorbidities of RA, more than 200 articles remained on this topic after screening 2000 abstracts. Additional articles were added that were found after the search date until May 1, 2014, by screening rheumatologic journals.

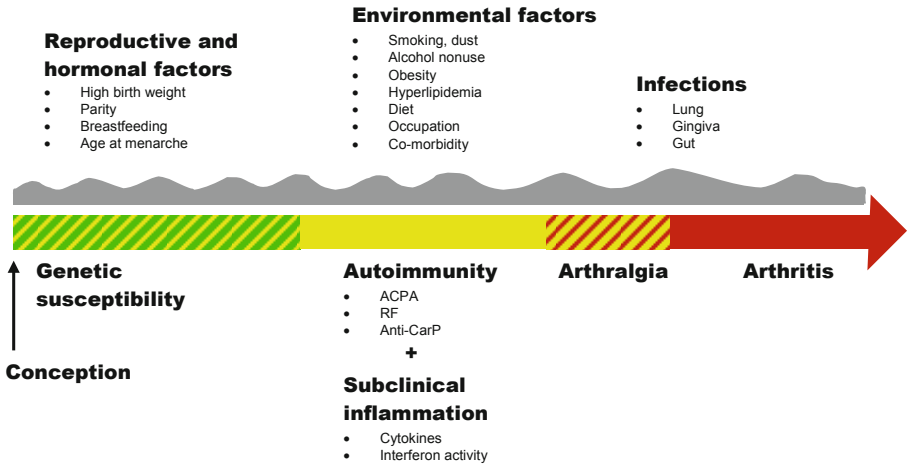

Fig. 1. The evolution of RA from health to disease
Abbreviations: ACPA, anti–citrullinated protein antibody; RF, rheumatoid factor. anti-CarP, anti-carbamylated protein antibodies.

RISK FACTORS: THE BUILDING BLOCKS OF PREDICTION

The current evidence on risk factors for RA is summarized in Table 1. Besides the factors reported in the table, many others have been investigated for their association with the risk of RA, but these studies have led to negative, inconclusive, or conflicting results. Among these are variables such as silicone implants[7–9]; consumption of coffee, tea,[10–13] or red meat[13–16]; geographic area[17–22]; and socioeconomic status.[23–28]

In contrast, some of the factors that have statistically significant associations with RA show opposite directions of risk in different studies. Examples of such cases are age at menarche, breastfeeding, and parity. This uncertainty makes the value of such variables questionable, even if they have been included in prediction models, as is the case with parity and breastfeeding in the model by Lahiri and colleagues.[29]

In conclusion, there are not many risk factors with strong and confirmed associations with RA. Among these are family history of RA, high birth weight, smoking, silica exposure, alcohol nonuse, obesity, diabetes mellitus, rheumatoid factor (RF), anti– citrullinated protein antibody (ACPA), and genetic variants such as the shared epitope (SE) and protein tyrosine phosphatase nonreceptor type 22 (PTPN22).

Table 1. Overview of evidence on risk factors for the development of RA

Risk Factor	Comments
Family history	Risk increases with number of affected family members[30–33]
	The longer the disease duration and the higher the age of the proband, the higher the risk[32]
	Some studies did not find an association between relatives with RA and risk of RA[33,34,35]
Genetic factors	Around 60 risk loci for RA are known, explaining 16% of total susceptibility[36]
	65% of RA risk is thought to be heritable[36]
Reproductive and hormonal factors	Risk is 2–4 times higher in women[37,38]
	A protective effect of oral contraceptives is suggested[38–43]
	High birth weight (more than 4 kg) increases risk[25,39]
	Lower risk during pregnancy, compensated by an increased risk in the first postpartum year[40,41]
	Complications during pregnancy may be related to a higher risk[42]
	Inconclusive or conflicting results for breastfeeding,[29,43–47] age at menarche, irregular menstrual cycles and age at menopause, postmenopausal hormone use,[43,44,48–50] lower testosterone levels,[37,51–53] parity, age at first childbirth,[29,40–44,50,54,55] low birth weight, and being small for gestational age[54,56,57]
Environmental factors	Smoking is the most established risk factor[58–61]
	Smoking interacts with the strongest genetic risk factor (HLA-SE) in a dose-dependent manner to increase the risk of seropositive RA[62]
	Alcohol consumption (even in small quantities) protects[63–65]
	High consumption of olive oil and fish (oil) protects[66–73]
	Inconclusive results were found for vitamin D intake and ultraviolet B exposure,[74–78] antioxidant and trace element intake,[16,68,70,71,79–87] and exposure to toxic elements[86,87]
Occupations and occupational exposures	Farmers, blue collar workers, and hairdressers are at increased risk[88–92]
	Silica exposure gives increased risk[90,93]
	Exposures that could not be related to RA: asbestos, mineral oil, organic dust, herbicides, insecticides, carbamates, organophosphates, carbaryl, glyphosate, malathion,[94–97] and ambient air pollution[98–100]
Infections and vaccinations	Frequent infections may predispose[54,55]
	One study reported increased risk after influenza vaccination[101]
	Risks could not be quantified for: Ebstein-Barr virus infection,[102] hepatitis C,[103,104] HIV,[105] Yersinia enterocolitica,[106] mycoplasma,[107] or Porphyromonas gingivalis infection of the gums,[108,109] and for immunization (other than influenza)[101,110–114]

table continues

Risk Factor	Comments
Comorbidities	Diabetes types 1 and 2[29,115] and inflammatory lung disorders[88,116–118] increase risk Schizophrenia is protective[119]
	Obesity and the related condition obstructive sleep apnea syndrome increase the risk[13,120–124]
	Dyslipidemia is present before RA and predicts RA[125–129]
	Other associations, such as for thyroid disease, are inconclusive[130]
Autoantibodies	Status and levels of (isotypes of) RF and ACPA associate with RA risk[131–143] Higher levels and the combination of RF and ACPA confer a higher risk[144,145]
	Additional predictive ability independent of RF and ACPA was shown for anti–carbamylated protein antibodies[146] and anti–peptidyl arginine deiminase type 4 antibodies[147]
Other biomarkers in blood	Several acute phase reactants and cytokines are increased in pre-RA or at-risk cohorts[1,148–162]
	TNF (receptor), cartilage oligomeric matrix protein, and a high interferon gene score are quantified risk factors[163,164]
Imaging	Ultrasonography abnormalities (mainly power Doppler signal) in seropositive patients with arthralgia were predictive of arthritis at the joint level in 1 study[165] and at the patient level in another study[166]
	Technetium bone scintigraphy is predictive of RA in patients with arthralgia[167] and can exclude inflammatory joint disease[168] Macrophage-targeted positron emission tomography predicts arthritis in ACPA-positive patients with arthralgia[169] The predictive capacity of MRI in arthralgia is not yet clear[170,171]
Symptoms	Predictive symptoms in combination with the presence of autoantibodies: duration <12 mo, intermittent symptoms, arthralgia in upper and lower extremities, morning stiffness 1h, self-reported joint swelling,[145] tenderness of hand or foot joints, and morning stiffness 30 min[166]

Abbreviations: ACPA, anti–citrullinated protein antibody; HIV, human immunodeficiency virus; MRI, magnetic resonance imaging; RF, rheumatoid factor; TNF, tumor necrosis factor.

PREDICTION RULES: PUTTING THE BLOCKS TOGETHER

In a manner similar to the way clinical characteristics, signs, and symptoms can be combined to diagnose a disease in a patient, the potential risk factors for a given disease can be combined by statistical modeling of variables measured in an at-risk population in order to produce prediction rules. The advantage of such models is that they clarify the relative impact of the individual variables and quantify the overall risk for individuals coming from that population. The validity of these models can then be further confirmed by testing them in other populations.

Recently, several prediction models have been published that attempt to quantify progression to RA (Table 2). Two of these models were based on large population studies, of which 1 was designed for investigating other diseases as well. One of these used clinical

characteristics to predict either seropositive or seronegative RA,[29] the other used the combination of clinical characteristics, autoantibodies, and a genetic risk score containing multiple genes (see Table 2 for the variables in the models).[172] Both studies achieve good prediction. However, it is uncertain whether these values can be reproduced in smaller populations.

Three other studies investigated the development of RA in ACPA-positive and/or RF-positive patients with arthralgia.[121,145,166] The patients were partly recruited in primary care, and partly in the rheumatology clinic. The models were based on clinical characteristics, symptoms, and antibody characteristics, in 1 study supplemented by ultrasonographic power Doppler signal (see Table 2).[166] All 3 models provide good discrimination between persons who do or do not develop RA. However, they require ongoing validation as other studies select and follow such cohorts of people at risk for RA. Similar studies from North America designed to predict RA in first-degree relatives of patients with RA are underway but have not yet gathered enough arthritis cases to enable the construction of prediction models.[149,173] These studies are hampered by the low frequency of autoantibodies or of increased biomarkers in relatives of patients with RA.

Measuring the risk of RA is also a matter of timing. During the early at-risk stage, before the onset of autoimmunity, clinicians can only measure genetic susceptibility and environmental factors (see the left part of Fig. 1). The predictive capability of models in this situation is becoming good, with areas under the curve of 72% to 77% for the prediction of ACPA-positive RA.[174] However, the measured risk is a lifetime risk, which makes it an abstract figure for the individual person at risk. Prediction including a time frame becomes possible nearer to the onset of clinical RA, when the aspects of symptoms, autoimmunity, and inflammation can be taken into account. In the Amsterdam risk model, points can be gathered for clinical characteristics, symptoms, and serology, with more points for high levels of ACPA or positivity for both ACPA and RF.[145] The more points, the higher the risk and the sooner the onset of arthritis can be expected (Fig. 2). This prediction reflects studies in pre-RA blood donors, in which autoantibody levels increase during the 1 to 3 years before the onset of clinical arthritis.[2,138] In an US cohort of 81 patients with clinical RA from whom stored serum was available from 1 to 12 years before disease onset, a biomarker profile including autoantibodies and cytokines was identified that predicts the imminent onset of clinical arthritis within 2 years.[160] Autoantibody epitope spreading by itself in the preclinical phase also predicts progression to classifiable RA.[143]

SCREENING STRATEGIES

Many medical, ethical, and economic issues need to be addressed before screening for risk of future RA can be offered to certain categories of unaffected persons. Basic requirements for screening groups of people to predict a disease are (1) a defined population to test; (2) the existence of an asymptomatic (or nonspecific symptomatic) phase; (3) the availability of a test with good accuracy, low rates of side effects, and low cost; and (4) the availability of a cost-effective intervention in the at-risk phase. Only the second requirement of an

asymptomatic phase is clearly fulfilled at present. Regarding items 3 and 4, no single test can identify those at risk for RA and no intervention exists with proven efficacy in the at-risk situation.[175,176] All efforts to predict RA and treat persons with an increased risk for RA are therefore currently regarded as investigational. The test for RA will eventually be a validated, cost-effective, and accurate prediction rule that is easy to apply. For comparison, consider the screening programs for colonic cancer, which have recently been established in several countries. All persons more than a certain age are offered screening, which leads to huge numbers of colonoscopies. The high cost of this procedure and the possibility of serious side effects need to be weighed against the benefit of removing polyps that would cause a high morbidity and mortality if left unnoticed.

Regarding item 1, careful consideration is needed to decide which population(s) should be screened or tested. The choices from general to specific are general population, relatives of patients with RA, persons with musculoskeletal symptoms, or persons with RA-specific autoimmunity. Because RA is not highly prevalent in most populations, with the possible exception of North American native peoples,[177,178] at this time it is not practical to test the general population for RA. Two recognizable target groups then remain: relatives of patients with RA and persons with musculoskeletal symptoms. The latter are found both in general practice and in rheumatology clinics. After history taking and physical examination, it must be decided which patients should proceed to further testing for RA risk, and which test to use. At present most clinicians use the RF and/or ACPA test, which are widely available and easy to perform. Except for patients with only RF positivity just above the reference range, the results give useful information. The question of who to test in general practice cannot accurately be answered at this time. This question requires structured longitudinal follow-up of patients in general practice, or the following of cohorts with clinically suspect arthralgia in rheumatology clinics.

Table 2. Prediction models of RA

First author and year (reference)	Cohort; Variables	Numbers	Results
Van de Stadt et al,[145] 2013	Seropositive patients with arthralgia Prediction rule variables: alcohol consumption, family history, symptoms <12 mo, intermittent, in upper and lower extremities, VAS 50, morning stiffness 1 h, swollen joints reported by patient, autoantbody status	Arthralgia: 374 (131 developed arthritis)	Prediction rule: AUC 0.82 (CI 0.75–0.89) Intermediate-risk vs low-risk group: HR 4.52 (CI 2.42–8.77) High-risk vs low-risk group: HR 14.86 (CI 8.40–28)
de Hair et al,[121] 2013	Seropositive patients with arthralgia Predictive variables: smoking and BMI	Arthralgia: 55 (15 developed arthritis)	Smoking (ever vs never) and risk of RA: HR 9.6 (CI 1.3–73) Obesity (BMI 25 vs <25) and risk of RA: HR 5.6 (CI 1.3–25))
Lahiri et al,[29] 2014	European Prospective Investigation of Cancer, Norfolk, United Kingdom 40–79 y Prediction rule variables: alcohol consumption, smoking, occupation, BMI, diabetes mellitus, parity	Total participants: 25,455 (184 developed IP, 138 developed RA)	Pack-years smoking in men and risk of IP: HR 1.21 (CI 1.08–1.37) Seropositive in men and risk of IP: HR 1.24 (CI 1.10–1.41) Having DM (I or II) and risk of IP: HR 2.54 (CI 1.26–5.09) Alcohol and risk of IP (per unit/d): HR 0.36 (CI 0.15–0.89) Overweight vs normal-weight and risk of seronegative IP: HR 2.75 (CI 1.39–5.46) Parity 2 vs no children and risk of IP: HR 2.81 (CI 1.37–5.76) Breastfeeding for every 52 wk and risk of IP: HR 0.66 (CI 0.46–0.94)
Sparks et al,[172] 2014	NHS, United States, females 30-55 y EIRA, Sweden, 18-70 y Prediction rule variables: family history, alcohol consumption, smoking, BMI, parity, autoantibody status, genetic risk score	RA cases: 1625 Controls: 1381	NHS Seropositive RA (model family history, epidemiologic, genetic): AUC 0.74 (CI 0.70-0.78) NHS Seropositive RA and positive family history: AUC 0.82 (CI 0.74-0.90) EIRA ACPA positive RA (model family history, epidemiologic, genetic): AUC 0.77 (CI 0.75-0.80) EIRA ACPA positive RA and positive family history: AUC 0.83 (CI 0.76-0.91) EIRA ACPA positive RA and positive family history, high genetic susceptibility, smoking and increased BMI: OR 21.73 (CI 10-44)

table continues

First author and year (reference)	Cohort; Variables	Numbers	Results
Rakieh et al, [166] 2014	Yorkshire, United Kingdom ACPA-positive patients with arthralgia Prediction rule variables: joint tenderness, morning stiffness ≥30 min, high-positive autoantibodies, positive ultrasonographic power Doppler signal	Arthralgia: 100 (50 developed RA)	Power Doppler model: Harrell's C 0.67 (CI 0.59-0.74) Progression to IA: Low risk (0 points) 0% Moderate risk (1–2 points) 31% High risk (≥3 points).62%

Abbreviations: AUC, area under the receiver operating characteristic curve; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; EIRA, Epidemiological Investigation of RA; HR, hazard ratio; IA, inflammatory arthritis; IP, inflammatory polyarthritis; NHS, Nurses Health Study; OR, odds ratio; VAS, visual analogue scale

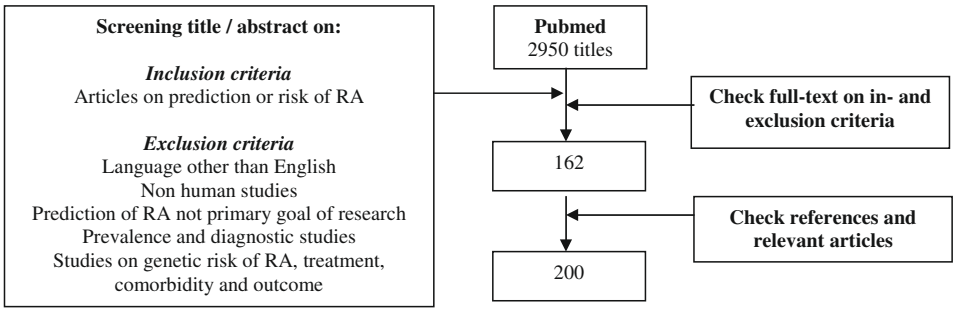

Fig 2. Flowchart search strategy

SUMMARY

There is a trend toward increasingly sophisticated prediction models for RA in different stages of risk. However, further work is needed to combine patient-level information with the published promising biomarkers into more robust models. For example, models for relatives of patients with RA, reflecting the early at-risk stage, depend largely on personal characteristics and genetic risk, whereas models for patients with arthralgia that reflect the late at-risk stage need to include patient-related and symptom characteristics in combination with biomarkers of autoimmunity and inflammation. In view of the vague and unspecific first symptoms of many patients who later develop RA, it will be necessary to better characterize and measure these symptoms in future models.[179]

However, because much is known about the risks for developing RA, it is already possible to use this information to design preventive interventions in persons at high risk for RA. At least in the late preclinical stage, several such interventions are currently being tested or planned.[180]

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CHAPTER

3

How does established rheumatoid arthritis develop, and are there possibilities for prevention?

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ABSTRACT

Established rheumatoid arthritis (RA) is a chronic state with more or less joint damage and inflammation, which persists after a phase of early arthritis. Autoimmunity is the main determinant of persistence. Although the autoimmune response is already fully developed in the phase of early arthritis, targeted treatment within the first months produces better results than delayed treatment. Prevention of established RA currently depends on the success of remission-targeted treatment of early disease. Early recognition is aided by the new criteria for RA. Further improvement may be possible by even earlier recognition and treatment in the at-risk phase. This requires the improvement of prediction models and strategies, and more intervention studies. Such interventions should also be directed at modifiable risk factors such as smoking and obesity. The incidence of RA has declined for decades in parallel with the decrease of smoking rates; however, a recent increase has occurred that is associated with obesity.

INTRODUCTION

The concept of "established rheumatoid arthritis" (RA) appears to be clear for the clinician. The picture arises of a patient with a "longstanding disease" that has caused a certain amount of joint and comorbid damage, and it remains in a fixed state with more or less active disease. The counterpart is the concept of "early (rheumatoid) arthritis," a more fluid state of recent synovitis where everything is still possible, including spontaneous or induced complete remission. Although the contrasting states are clear, the transition between them is gradual and less well-defined. It is reasonable to expect that causative factors for RA also influence the course of the disease, in this case the progression from early to established RA. For example, anti-citrullinated protein antibodies (ACPAs) are associated with both the risk of developing RA and the risk of a severe, unremitting course of RA.

In this chapter, we review risk factors for the development of early RA and for the transition to established RA. The concept of undifferentiated arthritis (UA) as a separate entity in a continuum from health to RA is undergoing changes due to new definitions. Finally, we focus on efforts to prevent RA from occurring (primary prevention) or from progressing from UA to RA (secondary prevention).

Apart from the uncertainty over the transitions between the different phases of RA, there is also considerable uncertainty over the question whether rheumatoid arthritis (RA) is a modern or an ancient disease. The name RA first appears in the medical literature in 1876[1], and the first unequivocal description of RA dates from 1631[2]. There is a scarcity of descriptions of the disease in Europe between 1700-1900[3]. This, combined with the fact that evidence of erosions compatible with RA has been found in ancient skeletons in North America, but not in Europe or the Middle East[4], has led to the suggestion that RA may be a communicable disease brought to the Old World after contact with the New World[1]. A good candidate factor for such an effect may be tobacco smoking, a habit imported from the New World that increased tremendously in the late 19th century followed by a decrease in the second half of the 20th century, roughly in parallel with changes in the incidence of RA.

RISK FACTORS FOR RA DEVELOPMENT

The risk of developing RA is determined by genetic susceptibility combined with environmental factors[5 6]. Certain environmental factors operate already early in life, and they may help to lay the foundation for autoimmunity. In a large part of those later developing seropositive RA, there is a phase of autoimmunity and subclinical inflammation, during which another transient cause of inflammation such as an infection is thought to trigger the onset of clinically apparent disease[7].

In the following, we present a short overview of genetic and environmental risk factors for RA, with a focus on recent publications. Due to the preclinical phase that many later

patients go through, biomarkers of autoimmunity and inflammation can also be used as risk factors or predictors of disease. Recently, several prediction models have been constructed using information from various cohorts of persons at risk of RA.

Genetic risk factors

Approximately 65% of RA risk has been shown to be heritable, and > 100 risk loci are now known. Most of these confer a low risk, and together they explain approximately 16% of total susceptibility[8]. It has become clear that ACPA-negative and ACPA-positive disease have a genetically different background[5 9]. The major histocompatibility complex (MHC) class II, DR beta 1 (human leukocyte antigen (HLA)-DRB1) alleles play a central role in the genetic risk of "seropositive" (ACPA and/or rheumatoid factor (RF)-positive) RA, mainly in patients who are ACPA positive[5]. Multiple alleles from this complex are associated with RA, which all share a region of similarity termed the shared epitope (SE). Besides these, several non-HLA genes have been identified. Most of the evidence comes from genome-wide association studies (GWAS)[10]. Until now, most GWAS investigating RA have been performed in seropositive individuals with a European background[10]. Recently, a review was published of specific genetic risk in Asian populations[11]. Although most single-nucleotide polymorphisms (SNPs) have the same effect sizes for developing RA in European and Asian people, some differences are found, mainly for PADI4 and PTPN22, which are more strongly associated with RA in Asian populations. Furthermore, the genetic risk in certain high-risk populations of North American Natives has been described, showing that most of the risk is conferred by a high prevalence of the SE in this population[12]. Evidence is lacking for many other populations. However, it seems that common SNPs found in ACPA-positive individuals with a European background also make individuals with a different ethnicity more susceptible to developing RA[9]. This was also shown, to a lesser extent, for ACPA-negative patients.

A disadvantage of the GWAS method is that the implicated SNPs are not necessarily causally linked to the development of RA itself. Moreover, until now, they cannot be used for individual prediction because of their low effect sizes. Most have odds ratios (ORs) for developing ACPA-positive RA of 1.1-1.2, with a few exceptions having an individual OR of around 2.0 (e.g., locus 1p13 on the PTPN22 gene, and 6p21 on the HLA*04 genes)[9].

Genetic risk scores

Given the many involved genes with small effect sizes, genetic risk scores (GRS) have been developed to help individual prediction of RA by adding up multiple validated genetic risk loci. In the next step these can be combined with environmental factors in prediction models. GRS for RA usually take both the number of alleles an individual possesses and the effect size of the alleles into account. Published GRS prediction models for RA, some including environmental factors, are presented in Table 1. In the case of multiple publications from one cohort, only the last publication is shown.

In summary, these studies show ORs of different models of around 2.0, and a wide variation of area under the curves (AUC) from a low value of 0.54 to a high value of 0.89

(with the highest values also including clinical parameters). A relatively high specificity for identifying individuals at risk (75-90%) is unfortunately accompanied by a very low sensitivity (30-45%). Therefore, apart from the disadvantage of its high cost, genetic risk prediction is thus still not precise enough to be used in current clinical practice, even though more and more genetic loci are being discovered. However, a recent study shows that a GRS plus environmental factors in family members of RA patients provides enough discrimination to enable the selection of high-risk subjects for intervention studies[13]. To support future research, Nagai et al. have made the open access database "RAvariome," which was developed to list all RA-associated genetic variants and check reproducibility over different ethnicities[14]. Their website (http://hinv.jp/hinv/rav/) also provides a "genetic risk predictor," which gives the lifetime risk on developing RA per individual. Unfortunately, as with the different GRS, the timing of RA development cannot be predicted by using this database.

Table 1. Prediction models for development of rheumatoid arthritis using genetic, clinical and behavioral (smoking) data

Reference	Cohort; variables	Numbers	Results
van der Helm 2010[15]	Early arthritis cohort, the Netherlands	570 UA	Model with genetic loci combined: AUC 0.54 (CI: 0.48-0.59)
	Genetic loci: HLA-DRB1 SE alleles, 11 SNPs		Genetic loci and clinical parameter: AUC 0.89 (CI: 0.86-0.95)
	Clinical parameter: smoking		
Kurreeman 2011[9]	EHR cohort, USA	1552 ACPA+RA	European ancestry: AUC 0.71 (CI: 0.68-0.73) African ancestry: AUC 0.63 (CI: 0.56-0.70)
	Genetic loci: 1 HLA allele, 29 SNPs	1504 controls	East Asian ancestry: AUC 0.74 (CI: 0.59-0.89) Hispanic ancestry: AUC 0.66 (CI: 0.56-0.76)
Scott 2013[16]	WTCCC and UKRAGG, UK	WTCCC/ UKRAGG:	HLA-SNP model WTCCC: AUC 0.80 (CI: 0.78-0.81) UKRAGG: AUC 0.76 (CI: 0.72-0.79)
	Genetic loci: 25 HLA alleles, 31 SNPs	1516/2623 RA	HLA-SNP-smoking model WTCCC: AUC 0.84 (CI: 0.81-0.87) UKRAGG: AUC 0.86 (CI: 0.80-0.91)
	Clinical parameter: smoking	1647/1500 controls	

table continues

Reference	Cohort; variables	Numbers	Results
Yarwood 2015[17]	Immunochip Consortium	Immunochip/	Genetic loci combined - Immunochip:
	Validation in CORRONA	CORRONA:	OR 2.0 (CI: 2.0-2.0), AUC 0.74, sens 35%, spec 91%
	Genetic loci: 45 SNPs, imputed amino acids at HLA-DRB1 (11, 71 and 74) and HLA-DPB1 (position 9) HLA-B (position 9)	11366/2206 RA	Genetic loci combined - CORRONA:
		15489/1863 controls	OR 2.0 (CI: 1.9-2.1), AUC 0.72, sens 30%, spec 92%
	Clinical parameters: gender, smoking		Addition of smoking improved the AUC to 0.80, without improving sens and spec
Sparks 2015[13]	NHS, USA (only females)	NHS/EIRA:	Genetic loci combined - NHS: AUC 0.62 (CI: 0.58-0.67)
	Validation in EIRA, Sweden	381/1752 RA	Genetic loci and clinical parameters: AUC 0.74 (CI: 0.70-0.78)
	Genetic loci: 8 HLA alleles, 31 SNPs	410/1361 controls	Genetic loci combined - EIRA: AUC 0.58 (CI: 0.55-0.60)
	Clinical parameters: family history, epidemiologic factors, HLA-smoking interaction		Genetic loci and clinical parameters: AUC 0.69 (CI: 0.67-0.72)

ACPA= anti-citrullinated protein antibodies, AUC= area under the receiver operating characteristic curve, CI= confidence interval (excluding 0,50 means statistically significant predictive value), CORRONA= Consortium of Rheumatology Researchers of North America registry, EHR= Electronic Health Records, EIRA= Epidemiologic Investigation of Rheumatoid Arthritis, HLA= human leucocyte antigen, NHS= Nurses' Health study, RA= rheumatoid arthritis, SE= shared epitope, sens= sensitivity, SNPs= single-nucleotide polymorhisms, spec= specificity, UA= undifferentiated arthritis, UK= United Kingdom, UKRAGG= RA Genetics Group Consortium UK, USA= united states of America, WTCCC= Wellcome Trust Case Control Consortium.

Environmental and behavioral factors

New risk factors for RA are being found, and systematic reviews have reevaluated established or controversial risk factors. The present situation is summarized in Table 2[5 6].

One controversial factor was alcohol consumption, which was shown earlier to be protective, even in small quantities[6]. Two reviews[18 19] confirmed this protective effect, although the effect size was small (summary ORs of 0.78 and 0.86, respectively), and one only found the effect in individuals later developing ACPA-positive RA. A nonlinear relationship was found in the dose-response meta-analysis. Lu et al. confirmed the finding that the association between alcohol and less development of RA was stronger in seropositive women[20].

Second, fish consumption (number of servings per week) was addressed in a systematic review[21]. This dose-response meta-analysis showed an inverse association between fish consumption of one to three servings per week versus never consumption and the risk of RA, with a relative risk (RR) of 0.76 (CI: 0.57-1.01) (not statistically significant). Third, the meta-analysis of the consumption of coffee and tea showed that only the use of coffee was related to RA development. The RR of total coffee intake was 1.3 for developing seropositive RA[22]. Fourth, much controversy exists about reproductive factors and sex hormone levels in both women and men in relation to RA. This holds true for menstrual cycle, parity, pregnancy, age at menopause, hormone use, and male testosterone levels. More recently published articles still show varying results, as also reflected in a recent review[23]. A publication that was not included in this review reported that pregnancy complications, namely preeclampsia, and poor self-rated health during pregnancy were related to a higher risk of later RA[24]. Baydoun et al. investigated reproductive history and postmenopausal RA, but only found menopausal age below 40 years to confer the risk of RA after menopause[25]. Moreover, no significant relationship could be found between the use of oral contraceptives and the development of RA in two reviews incorporating a total of 28 studies[26]. Two other publications produced conflicting results of testosterone levels in men. One did not show a difference between testosterone levels in pre-RA cases versus controls[27] and the other found lower testosterone levels before the diagnosis of RF-negative RA[28]. Finally, a recent article publishes information about geographic area and RA incidence, and prevalence and mortality rates[29]. Although the focus was more on the burden of disease, the authors do present data showing that RA is more prevalent in Northern countries as compared with countries near the equator.

More focus has been directed lately toward different dietary components and the risk of RA development. Already in 2004 a review suggested the possible role of diet, but it could not quantify the risk[30]. Recent publications have focused more on different types of diet. No significant relations could be found for a Mediterranean type diet[31], a carbohydrate-restricted diet[31] and sodium intake (which only led to a significantly increased risk when combined with smoking)[32]. Interestingly, sugar-sweetened soda consumption ≥1 serving/day (compared with <1 serving/month) was significantly related to the development of both seropositive and late-onset-seropositive RA (age after 55 years) in women with hazard ratios (HRs) of 1.63 and 2.62, respectively (corrected for other lifestyle components)[33]. The amount of added sugar in these drinks may contribute to the pathogenesis of RA by inducing obesity, insulin resistance, and inflammation. In light of the recent rise in obesity prevalence and RA incidence (see subsequently), this might be an important point of interest, and suggests a possibility to intervene in the at-risk subjects.

Most environmental risk factors seem to be more related to seropositive than to seronegative RA. However, obesity was shown to be related mainly to seronegative RA in most publications[5 34 35], with only one report also showing a higher risk of ACPA-positive RA in women[36]. All underline the importance of obesity as a risk factor. As obesity may be in part related to little exercise, it was hypothesized that regular exercise protects against RA. This was confirmed by two studies which showed regular physical activity indeed leads to

less RA, and, if it did occur, patients presented with milder disease[37 38].

Besides obesity several other comorbidities have since long been linked to the development of RA, such as diabetes mellitus and schizophrenia. Recently, two other diseases have been investigated. Sleep disorders (without sleep apnea) had an HR of 1.45[39] and autoimmune thyroid disease was seen more frequently in RA cases than in controls (together with more thyroxin substitution before RA development)[40].

The exact mechanism as to how systemic autoimmunity advances into local inflammation in the joints still needs to be further investigated[7]. It is thought that infections may trigger the onset of clinically apparent disease. Some recent publications have focused on the presence of infections before RA onset and specific pathogens. Prior infection-related medical visits and bacterial colonization are shown to predispose the development of RA, mostly in the year preceding diagnosis[41 42]. However, another study found a decreased risk of gastrointestinal and urinary tract infections and no relation for other infections[43]. So far, no specific pathogen could be quantitatively linked to RA development[6]. Regarding the related subject of vaccination, only one out of many studies reported an increased risk <1 year after tetanus vaccination[44].

Finally, de Roos et al. investigated living in the proximity to traffic, ambient air pollution, and community noise. They found a higher risk of RA when living within 50 m from the highway (OR 1.37), but they could not relate this to ambient air pollution or noise[45]. In this study, it is good to note that it was not possible to correct for confounding factors such as low social economic status, nonwhite race, and smoking. Therefore, the results may be biased. Besides, another study could also not find a relationship between air pollution and the development of RA[46].

A distinction was made between traditional risk factors, meaning generally accepted risk factors before at least 4 years ago (most already presented in previous edition of Best Practice & Research), and the ones receiving more attention over the past years and generating new insights.

Table 2. Environmental risk factors for development of rheumatoid arthritis

Traditional risk factors **Validated risk factor**	**Comment**
Family history	65% of RA risk is thought to be heritable
Female gender	Females have 2-4 times higher risk
Ageing	Onset usually around sixth decade of life
Smoking	One of the main risk factors, dose-dependent risk effect
Lower education level	Possibly linked to lifestyle or certain occupations
Silica exposure	Industrial exposure: mining, construction, agriculture, electronics
Pregnancy	Increased risk in the year after childbirth

table continues

High birth weight	> 4 kg
Fish oil, olive oil	Protective effect; believed to have anti-inflammatory properties
Comorbid conditions	Diabetes mellitus type 1 and 2, inflammatory lung diseases, dyslipidemia. Schizophrenia (protective)
New risk factors or new information on known risk factors	
Suggested risk factor	**Comment**
Sugar-sweetened soda	May induce obesity, insulin resistance and inflammation
Obesity	Conflicts about whether it increases risk of both seronegative and seropositive RA
Physical activity	Associated with less and milder RA
Infections	Frequent infections may predispose, although some contradict this finding, no specific pathogens causally linked to RA
Sleep disorders	The non-apnea types show higher RA rates later on
Autoimmune thyroid disease	Subsequent RA seems more frequent
Tetanus vaccination	One study reported increased risk <1 year after vaccination
Recent reviews	
Alcohol consumption	Protective effect, mainly for seropositive RA
Fish consumption	No significant relationship with RA development
Coffee consumption	Coffee consumption gives a higher risk of seropositive RA
Reproductive/hormonal factors	Controversy continues
Use of oral contraceptives	No significant relationship with RA development
Geographic area	RA is more prevalent in Northern countries as compared to countries near the equator
Inconclusive/conflicting results	
Age at menarche and menstrual cycles, parity and age at first childbirth, breastfeeding, oral contraceptives, postmenopausal hormone use	
Periodontitis	
Previous blood transfusion	
Consumption of coffee and tea, red meat	
Ultraviolet B exposure and vitamin D levels, antioxidant and trace element intake, exposure to toxic elements and air pollution	
Silicone implants	

Gene-environment interactions and environmental factors influencing each other

A strong interaction exists between smoking and genetic background (namely HLA-DRB1 alleles)[10]. Besides, smoking interacts with autoantibody-positive status, gender (higher influence in males), and consumption of dietary sodium[5 32] to a lesser extent. Furthermore, adding positive family history of RA to genetic risk models increases the predictive capacity. However, in general, the gene-environment interactions add too little information to the models to be of clinical use[13].

Autoimmunity and biomarkers

Approximately two-thirds of RA patients test positive for RF and/or ACPA at diagnosis, underlying their importance in this disease. Other antibodies preceding and predicting

a diagnosis of RA, independent of RF and ACPA status, are anti-carbamylated protein antibodies and anti-peptidyl arginine deiminase type 4 antibodies[6]. The discovery of new related autoantibody systems may in the future give more insight into the pathogenesis of RA.

Other blood-based biomarkers such as acute phase reactants or cytokines were not found to have predictive capacity for RA[6].

Clinical prediction models
Quantifying progression to RA with genetic modeling alone is not ready for clinical use, as we have shown earlier. Several studies have taken a different approach by using a combination of clinical characteristics, symptoms, and sometimes imaging findings. The resulting prediction rules are summarized in Table 3. Validation is still needed for all models. With this restriction, they can be useful to inform persons with musculoskeletal symptoms about their risk of arthritis/RA, especially in the presence of RA-related antibodies.

CHANGING INCIDENCE RATES AND MODE OF PRESENTATION OF RA

In 1979, it was hypothesized that RA as a disease entity would disappear eventually [51]. Currently, more evidence exists of a pattern of rises and falls over the decades. Over the first half of the 20th century, no data are available. Alamanos et al. summarized studies on incidence and prevalence rates of RA (according to the 1987 American College of Rheumatology (ACR) criteria) from the second half of the 20th century[52]. Two out of the three studies, which evaluated time trends of RA occurrence, reported a declining RA incidence of 15% and 47% in 1 and 4 decade(s), respectively (1980-1990 in MN, USA, and 1955-1994 in Finland). In Greece, the incidence remained stable between 1987 and 1995. Studies in Japan and of North American Natives in the USA have also noted a declining incidence of RA[53 54]. The decline in incidence combined with a shift toward higher age at the onset of disease has been attributed to a so-called birth cohort effect[55]. This is a term used in social science to describe characteristics of an area of study over time among individuals who are defined by certain early life influences. Following generations will benefit or be harmed by these influences of their ancestors, in this case leading to a decline in RA incidence. However, which specific risk factors would be implicated in the decline of the incidence has not been specified.

Table 3. Clinical prediction models for development of rheumatoid arthritis

First author and year (ref)	Cohort; variables	Numbers	Results
van de Stadt 2013[47]	Seropositive arthralgia patients Prediction rule variables: alcohol nonuse, family history, several symptoms, autoantibody status	Arthralgia: 374 (131 developed arthritis)	Prediction rule: AUC 0.82 (CI: 0.75-0.89) Intermediate vs low risk group: HR 4.52 (CI: 2.42-8.77) High vs low risk group: HR 14.86 (CI: 8.40-28)
de Hair 2013[48]	Seropositive arthralgia patients Predictive variables: smoking, BMI	Arthralgia: 55 (15 developed arthritis)	Smoking (ever vs. never) and risk of RA: HR 9.6 (CI: 1.3-73) Obesity (BMI ≥25 vs. <25) and risk of RA: HR 5.6 (CI: 1.3-25)
Lahiri 2014[49]	European Prospective Investigation of Cancer, UK Prediction rule variables: alcohol use, smoking, occupation, BMI, diabetes mellitus, parity	Total participants: 25455 (184 developed IP, 138 developed RA)	Pack-years smoking in men and risk of IP: HR 1.21 (CI: 1.08-1.37) Seropositive in men and risk of IP: HR 1.24 (CI: 1.10-1.41) Having DM (I or II) and risk of IP: HR 2.54 (CI: 1.26-5.09) Alcohol and risk of IP (per unit/day): HR 0.36 (CI: 0.15-0.89) Overweight and risk of seronegative IP: HR 2.75 (CI: 1.39-5.46) Parity ≥2 and risk of IP : HR 2.81 (CI: 1.37-5.76) Breastfeeding and risk of IP: HR 0.66 (CI: 0.46-0.94)
Rakieh 2014[50]	ACPA-positive arthralgia patients Prediction rule variables: several symptoms, high-positive ACPA, positive ultrasound power Doppler signal	Arthralgia: 100 (50 developed RA)	Power Doppler model:Harrell's C 0.67 (CI: 0.59-0.74) Progression to IA:Low risk (0 points) 0% Moderate risk (1–2 points) 31% High risk (≥3 points) 62%

ACPA= anti-citrullinated protein antibody, AUC= area under the receiver operating characteristic curve, BMI= body mass index, CI= confidence interval, DM= diabetes mellitus, EIRA= Epidemiological Investigation of RA, HR= hazard ratio, IA= inflammatory arthritis, IP= inflammatory polyarthritis, NHS= Nurses' Health Study, OR= odds ratio, RA=rheumatoid arthritis, VAS= visual analogue scale. Reproduced from Turk et al, 2014[6].

Another important note about changes in incidence rates over time is that the timing of the measurement and used RA criteria can vary between studies, and it also depends on the duration of the study period, mode of presentation, awareness of the disease by general practitioners, and the delay of referral after symptom onset. In the following, we describe two of these factors in more detail. First, the new ACR/European League Against Rheumatism (EULAR) 2010 criteria for RA (see subsequent discussion) are more sensitive than the earlier criteria, which will probably lead to earlier detection (and treatment) and thereby affect the measurement of incidence rates in the coming years[59]. Second, within Europe, the variation in the delay of first assessment of RA patients is substantial, with a median range of 16-38 weeks per center and a difference at its highest of 34% in seeing patients within 12 weeks of symptom onset[60]. This could partly explain differences of changes in incidence rates across European countries, and even less is known about such a variation outside Europe.

In conclusion, relevant trends are a steady decrease of worldwide RA incidence during the period 1955-1995, followed by a recent increase in at least Denmark and the USA, probably explained in part by changing environmental factors. Furthermore, factors such as differences in the use of RA criteria and differences in the awareness of RA across countries can affect the incidence rates over time.

UA, PAST AND PRESENT

The term "UA" suggests that the condition in the patient concerned is in a stage of transition from an unspecified type of arthritis toward either RA, another arthritis-associated diagnosis, or spontaneous remission. The incidence of UA ranges from 41 (in Finland) to 149 (in Sweden) per 100.000 adults, and 13-54% of these patients will eventually develop RA, according to the 1987 ACR criteria[61]. In the past, the transition from UA to RA was equivalent to fulfilling the 1987 ACR criteria for RA[62] after a phase with arthritis in which these criteria were not yet fulfilled. In practice, this mainly applied to the progression from oligoarthritis to polyarthritis and/or the development of erosive disease, as other elements of the criteria set such as RF or nodules do not often appear in early arthritis, if not present at the first presentation[63]. Therefore, the transition from UA to RA could be viewed as the development of a more severe arthritis in inadequately controlled early RA, which made this an outcome of interest. The main predictor of the transition was the ACPA status of the patient[64].

The 2010 ACR/EULAR criteria for RA aim to increase sensitivity in early disease[65], which is mainly achieved by a focus on small joint involvement and serology. Thus, a patient with one swollen finger joint of 6 weeks duration and a high-titer ACPA will already classify as RA. The consequence is that the subgroup of UA in early arthritis patients is strongly reduced, and it is now composed mainly of seronegative (oligo-) arthritis. On average, these "2010 UA" patients will have a milder and more heterogeneous disease than "1987 UA" patients[66]. Although both the 1987 and 2010 criteria for RA are classification and not diagnostic criteria, the 2010 criteria were specifically developed for use in early disease,

and they reflect the trend among clinicians to diagnose RA earlier and even in the presence of only a few involved joints.

Just as was the case with 1987 UA patients, a part of 2010 UA patients will remit and a part will go on to have a severe disease course. In a recent study of three early arthritis cohorts, the Leiden prediction rule (developed to predict 1987 RA in 1987 UA patients) and the ACPA status failed to predict the development of 2010 RA in 2010 UA patients[67]. New biomarkers are needed that can help to detect the 2010 UA patients at high risk of disease progression, so that they may be considered for more aggressive therapy than the remaining UA patients, for whom symptomatic treatment may be sufficient. An example is anti-CarP antibodies, which were shown to predict radiographic damage in early ACPA-negative RA patients[68]. Next to blood-based biomarkers, imaging modalities such as ultrasound or magnetic resonance imaging (MRI) may prove to be useful in this respect[69 70].

WHEN DOES EARLY RA BECOME ESTABLISHED RA?

This question gives rise to the suggestion that there is a difference between the pathology at the beginning of the disease and what is found later on, and that this distinction has clinical significance. In fact, this is closely related to the concept of a therapeutic "window of opportunity," which states that treatment initiated at an early stage of the disease is more successful than when it is started later on. "Early" would mean that there is joint inflammation of recent onset, which may at this stage still resolve without further consequences or at least decrease to a barely detectable minimum, if treated adequately. "Established" on the other hand would mean the inflammation is there to stay, more or less pronounced, whatever intervention is applied. Moreover, the concept of "established" RA will generally include damage to the joints, and diverse comorbidities with their complications such as osteoporosis or cardiovascular disease, which may arise as a consequence of the ongoing inflammation.

To begin with, the pathology of RA does not suddenly start around the onset of clinical arthritis. RA-specific systemic autoimmunity as well as nonspecific subclinical inflammation occurs in concert on average 5 years before the onset of symptoms[71 72]. During the period of presymptomatic autoimmunity, there is a maturation of the immune response to citrullinated and carbamylated antigens, which is consistent with an increasing break of tolerance[73]. Thus, the number and levels of different ACPA specificities increase toward the onset of arthritis; however, there is no further increase once clinical arthritis has begun[74]. Accordingly, the number and type of ACPA specificities do not differ largely between early and late disease[75]. Anti-immunoglobulin G (IgG) antibodies or RF arise later and less frequently than ACPA, and they may continue to increase in prevalence after the onset of arthritis[74 76].

The synovial infiltrate of knee joints of RA patients that had not been clinically swollen before, nevertheless, showed chronic inflammation[77]. In animal models of RA, inflammation in joint pathological specimens precedes clinically detectable inflammation.

Persons at an increased risk of RA have increased numbers of T-cells in their knee synovium even if they did not yet have knee symptoms[78], again suggesting that the transition to chronic inflammation takes place before the onset of clinically apparent arthritis. Once the symptoms begin, a higher number of recognized ACPA specificities are associated with a higher rate of transition to clinical arthritis[73]. This means that once a person notices the first symptoms of RA, the pathological immune response has matured to a large extent, but not completely.

Although the immunological driving processes of RA do not seem to differ between early and late RA, it is well known that better clinical results can be obtained by treating RA patients early and aggressively[79]. A recent analysis concluded that this window of opportunity starts to close 4 months after the onset of symptoms[80]. This implies it is still possible during that period to interrupt certain processes perpetuating the chronicity of inflammation. One of these could be the total burden of inflammation, which builds up in the early clinical phase. It is conceivable that once a critical mass of inflammatory tissue has been reached, it is no longer possible to control it effectively. This theory is difficult to test, as there is no technique available at present, which can reliably test the total load of inflammatory tissue in a person.

PRIMARY PREVENTION OF RA

The different stages of RA development offer opportunities for preventive interventions, varying from (primary) prevention of the development of arthritis in the at-risk phase to (secondary) prevention of progression from UA to RA or from early to established RA.

The list of risk factors for RA (Table 2) shows that there are several opportunities for lifestyle changes to help prevent RA. Smoking is the strongest environmental risk factor for RA, in particular for ACPA-positive RA, and it has been calculated in Denmark and Sweden that population-wide cessation of smoking would result in more than one-third less cases of ACPA positive RA[81 82]. Other potentially modifiable factors include dietary changes, weight reduction and dental care to reduce periodontitis. These are currently being addressed in the *PRE-RA Family Study Boston,* which is exploring the attitudes of family members of RA patients toward a lifestyle intervention based on a genetic plus environmental risk assessment[83]. Participants are randomized to receive feedback and education concerning their personalized RA risk based on demographics, RA-associated behaviors, genetics, and biomarkers or to receive standard RA information. Four behavioral RA risk factors are included in the risk estimate: smoking, excess body weight, poor oral health, and low fish intake. The trial outcomes will be changes in willingness to alter behaviors. As we learn more about these relations, such information programs can be refined. At present, the most important advice is for family members of ACPA-positive RA patients, to refrain from smoking[82].

The concept of primary prevention of RA with drugs has become possible through the recognition of a prolonged at-risk phase with variable symptoms and/or autoimmunity

before the outbreak of clinical RA. The first clinical trial was a post hoc analysis of the effect of vitamin E in a study designed to prevent coronary heart disease in the general population[84]. Although the trial was negative the for prevention of both heart disease and RA, there was a trend toward protection against RF-positive RA. The next study was a trial of two intramuscular injections of 100 mg dexamethasone or placebo in ACPA and/or RF-positive arthralgia patients[85]. Furthermore, this trial did not affect the onset of arthritis, although autoantibody levels were suppressed for 6 months. Meanwhile, trials of rituximab (Prevention of RA by B cell-directed therapy (PRAIRI) trial, NTR1969; www.trialregister.nl), of abatacept (Arthritis Prevention In the Pre-clinical Phase of Rheumatoid Arthritis (APIPPRA) trial; www.isrctn.com/ISRCTN46017566) and of atorvastatin (STAtins in the Prevention of RA (STAPRA) trial; NTR5265; www.trialregister.nl) in the same patient category are ongoing.

Some clinicians confronted with seropositive arthralgia patients will try antimalarial treatment. Apart from being a relatively nontoxic RA remedy, the rationale for this treatment comes from the experience with antimalarials in the treatment of palindromic rheumatism, a rather ill-defined syndrome of intermittently occurring peripheral arthritis. A subgroup of those patients is RF- or ACPA-positive with a tendency to develop RA[86], and this tendency was found to be markedly reduced in a retrospective survey in those taking antimalarials[87]. Another retrospective study reported a marked reduction in frequency and duration of attacks in palindromic rheumatism patients taking chloroquin[88].

In conclusion, no intervention has yet showed an effect in a randomized controlled trial in the primary prevention of RA. The scarcity of data gives rise to the suggestion that it is not easy to perform clinical trials in the at-risk phase of RA, and that positive outcomes are not readily obtained. A major ethical issue with intervening pharmacologically in this phase, is that persons are exposed to potentially toxic drugs, whereas a part of the study subjects will never develop RA.

SECONDARY PREVENTION OF RA

One of the explicit goals of the 2010 ACR/EULAR criteria for RA was to facilitate the performance of trials in early RA[65], in order to make even better use of the window of opportunity in early disease. The underlying idea was that it would be easier to design a trial for patients who were classified as RA instead of as UA. Nevertheless, already before the publication of the 2010 criteria, a number of trials had been conducted with the intention to prevent the progression of early disease, mostly not classifying as RA according to the 1987 ACR criteria[62]. Part of the outcome measures of these trials was a reduction of the transition of UA to RA, which means that a successful outcome could be regarded as secondary 1987 ACR criteria prevention of RA.

The results of the PROMPT study of methotrexate to prevent progression of UA to RA (1987 criteria) and its long-term follow-up showed less progression to RA, but only in ACPA positive patients and only as long as the treatment was continued[89]. Other trials in early

oligoarthritis or UA have noted some transient benefit from treatment with intramuscular (STIVEA trial) or intraarticular corticosteroids compared to placebo or nonsteroidal anti-inflammatory drugs[90 91]. However, the Stop Arthritis Very Early (SAVE) trial observed that the development of 1987 RA was not delayed by intramuscular glucocorticoid treatment in oligoarthritis patients[92].

Biologics have also been tested for this indication. Three months of infliximab did not prevent progression to 1987 RA after 1 year[93]. Six months of abatacept slightly reduced the progression of UA to 1987 RA from 67 to 46%[94]. Abatacept treatment also had an impact on radiographic and MRI inhibition, which was maintained for 6 months after treatment stopped. The STREAM study, a trial of aggressive treatment including adalimumab aimed at remission versus usual care in oligoarthritis patients, did not show a better outcome for aggressive treatment, although there was a trend toward less radiographic damage in the aggressively treated group[95]. In a larger two-step study aiming at early remission of early oligoarthritis or RA (IMPROVED study), similar rates of remission were achieved after 6 months of 61%. Of those not in remission at 6 months, more patients achieved remission at 12 months with adalimumab than with conventional disease-modifying antirheumatic drug (DMARD) combination therapy[96].

In conclusion, intervening in the early phase of clinical arthritis with minimal joint involvement leads to similar remission rates as are found in early RA, and there is not much evidence supporting the halting of progression from UA to RA. This suggests that it is not easy to further enhance the benefit of treating RA patients early, by treating patients with fewer involved joints even earlier in the disease course.

The broader question to what extent the transition to established RA can be prevented in patients with early RA is answered by the relative but not yet absolute success of early targeted treatment during the window of opportunity. Secondary prevention in this case could be defined as the goal of achieving and maintaining remission by early and aggressive treatment followed by minimization of therapy[97]. Spontaneous remission occurs frequently in early arthritis, especially seronegative arthritis, and only rarely in established RA (Fig. 1)[61]. Patients who achieve early remission can sometimes maintain their remission for prolonged periods after stopping medication[98]. For patients with established RA in remission, it is less often possible to maintain a drug-free remission[99 100]. Taken together, it appears that DMARD-free remission can occur (13-50%), and it is not so rare as previously thought (4-6%). At any rate, there is hope that by achieving early remission with aggressive therapy, the disease can be controlled with less total medication in the long run than with milder treatment regimens.

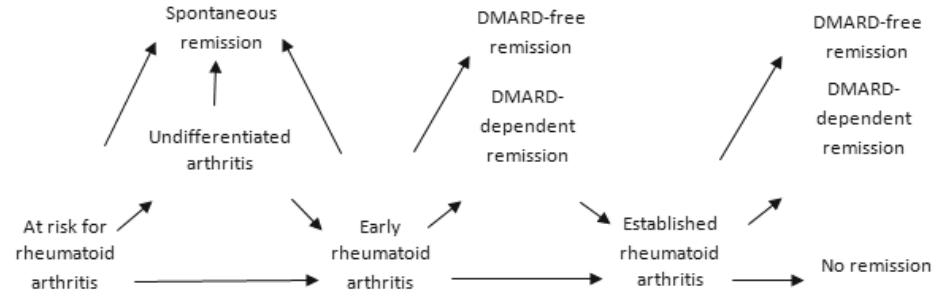

Figure 1. Remission in different stages of rheumatoid arthritis

SUMMARY

The increasingly successful management of RA now leads to more patients achieving early and sustained remission, and this will lead to less patients progressing to the classical state of established RA. A next goal in the management of RA can be the improved recognition and intervention in the early or even at-risk phase of RA.

Prediction depends on the knowledge of risk factors. Recent advances in the risk factor assessment of RA include alcohol consumption as a confirmed protective factor, whereas fish consumption could not be confirmed as a protective factor. New risk factors are coffee consumption, sugar consumption, sleep disorders, and thyroid disease, whereas exercise and recent infections have been put forward as protective factors. Increasingly, risk factors are being combined to establish prediction rules. Those containing genetic risk plus environmental factors are not yet ready for general use. However, new prediction rules for arthralgia subjects using clinical characteristics, serology, and sometimes imaging are quite simple to perform, and they can be used to inform patients of their risk of RA.

Interestingly, RA incidence seems to have been declining since 1955, when formal measurements started, at least until the end of the last century. However, recent reports suggest that the incidence is on the rise again, mainly in seronegative females, and that this can be ascribed largely to the recent increase in obesity. When comparing trends in different countries, it becomes necessary to take into account the large variation between countries in the public and physician awareness of the need to identify RA early.

The problem of assessing UA has been reduced considerably by the introduction of the 2010 RA criteria. Many former UA patients can now be classified as RA, leaving a smaller group of UA patients with more heterogeneous and milder disease. Treating UA patients early gives results similar to early treatment of RA. In line with the concept of an early "window of opportunity," a few studies have attempted to treat patients at an even earlier stage, before clinical arthritis becomes apparent. These primary prevention studies with pharmacological interventions have not yet produced positive results. Although these

efforts are continued, the identification of modifiable risk factors for RA such as smoking, obesity, and lack of exercise should incite physicians to promote healthy behavior in persons at risk of RA.

PRACTICE POINTS

- Worldwide RA incidence showed a steady decrease during the period 1955-1995, followed by a recent increase in at least Denmark and the USA.
- New possible risk factors for the development of RA are non-alcohol use, coffee consumption, sugar-sweetened soda intake, obesity, physical inactivity, sleep disorders, and thyroid disease.
- Possible options for primary prevention of RA include dietary changes, weight reduction and dental care. No drug intervention has proven to be effective in the prevention of RA.
- With the advent of the 2010 ACR/EULAR criteria, the subgroup of UA in early arthritis patients is strongly reduced, and it contains mainly seronegative (oligo-) arthritis patients with a mild disease course.
- Secondary prevention of RA is becoming less of an issue due to the high sensitivity of the 2010 ACR/EULAR criteria in early disease, and the tendency to treat early arthritis rapidly.

RESEARCH AGENDA

- Improve prediction models of RA by integrating personal characteristics, symptoms and genetic information with new biomarkers.
- Establish simple prediction aids for different situations, for example, in the general practitioner (GP) office, the rheumatology clinic, or the general public.
- Controlled intervention studies in persons at risk of RA in different stages.
- Improved identification of UA with poor prognosis.

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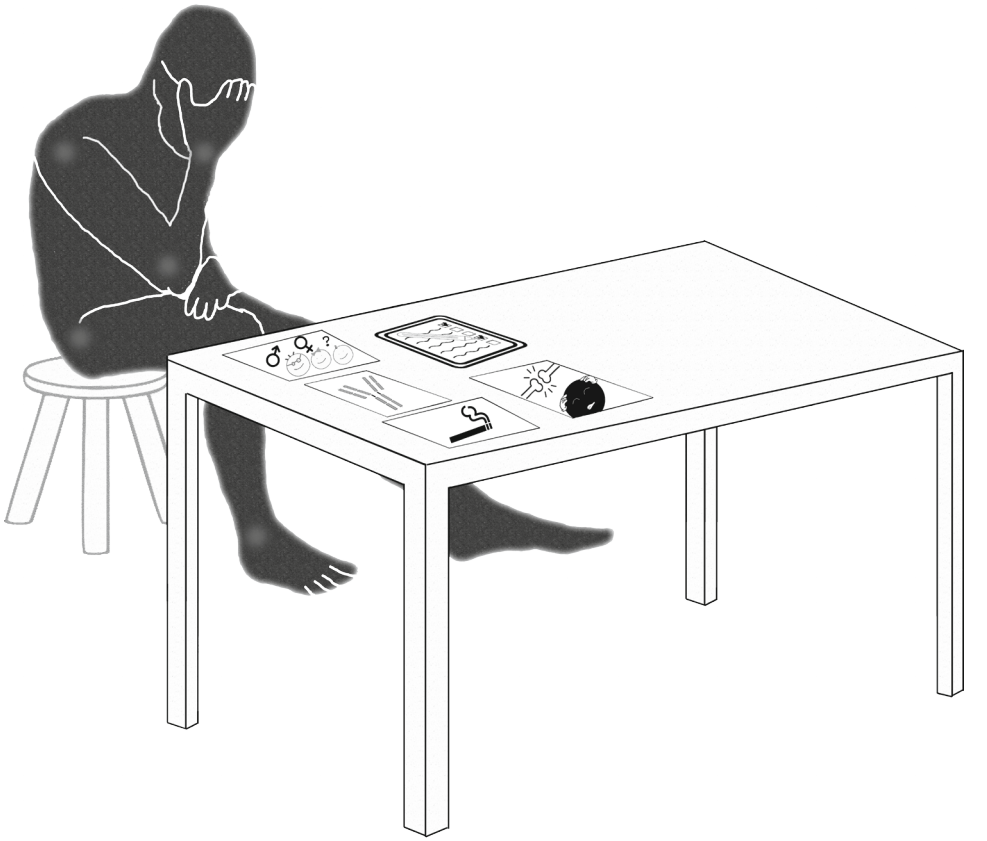
PART II

Symptoms in seropositive arthralgia patients and in primary care



CHAPTER 4

Depressive mood and low social support are not associated with arthritis development in seropositive arthralgia patients, although they predict increased musculoskeletal symptoms

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ABSTRACT

Objective. Studies on the role of psychosocial vulnerability in the development of arthritis must be performed early in the disease course to exclude the reverse explanation that arthritis leads to psychological symptoms. Therefore, the objective of this study was to investigate the longitudinal (5-year) association between depressive mood, daily stressors, avoidance coping, and social support as predictors, and the development of arthritis and other clinical parameters as outcomes, in persons with seropositive arthralgia at risk of developing RA.

Methods. Five-year follow-up data of 231 patients from the Reade seropositive arthralgia cohort were used. Clinical and psychological data were collected using physical examinations and questionnaires. Mixed models and Cox regression analyses were used to assess the 5-year associations between depressive mood, daily stressors, avoidance coping or social support, and the development of arthritis or clinical parameters (tender joint count, VAS pain, VAS morning stiffness, ESR).

Results. Higher scores for depressive mood and lower scores for social support were not associated with the development of arthritis nor with ESR. However, they were longitudinally associated with an increase in pain ($P<0.001$), morning stiffness ($P<0.01$) and tender joint count ($P<0.001$). No consistent associations were found between daily stressors, avoidance coping and the development of arthritis or other clinical parameters.

Conclusion. Although an effect on the development of arthritis could not be demonstrated, a strong longitudinal association was found between high depressive mood, low social support and clinical parameters. In persons with seropositive arthralgia, depressive symptoms and low social support may increase musculoskeletal symptoms.

KEY MESSAGES

What is already known about this subject?

- Depression, daily stressors, avoidance coping and lack of social support have been linked to disease activity in persons with RA, however results of longitudinal studies on these relationships are inconsistent or scarce.

What does this study add?

- This is the first study that explored the predictive roles of depressive mood, daily stressors, avoidance coping, and social support in the development of physician-diagnosed clinical arthritis in persons at risk of developing RA without overt inflammation.
- In persons with seropositive arthralgia, an effect of depressive mood, daily stressors, avoidance coping, and social support on the development of clinical arthritis could not be demonstrated.
- However, a strong longitudinal association was found between high depressive mood, low social support and pain, morning stiffness and tender joint count.

How might this impact on clinical practice?

- The results indicate that in persons with seropositive arthralgia, depressive symptoms and low social support may increase musculoskeletal symptoms.

INTRODUCTION

Depression is highly prevalent in persons with rheumatoid arthritis (RA). A meta-analysis of studies reporting prevalence estimates for depression in RA revealed rates between 15% and 40%, depending on the manner in which depression was measured[1]. Depression is also common in diseases that are associated with RA through shared pathogenic factors, such as cardiovascular diseases and diabetes mellitus[2 3], and may even increase the risk of developing these diseases[4 5]. It seems that depressed persons also have an increased risk of developing RA, however this risk is less well established[6]. Likewise, post-traumatic stress disorder (PTSD) has been associated with an elevated risk of the development of RA and increased RA disease activity[7-10], and perceived stress has been associated with self-reported arthritis development 3 years later in women[11]. In addition, studies point to an unfavorable course of disease activity in RA patients with symptoms of depression[12 13]. The same holds true for avoidance coping and lack of social support, which also have been associated with increased disease activity in persons with RA[14 15]. Finally, besides indications for an unfavorable disease course, it has been shown that persons with RA and depression generally have reduced treatment response and poorer health status, with as a result an increased risk of mortality and work disability already at an early stage of RA[16-18]. Several mechanisms have been described that may partially explain the increased cardiovascular risk in persons with depression, including unhealthy behaviors, pathophysiological dysregulations (immune system, hypothalamic–pituitary–adrenal axis (HPA-axis) and metabolic), shared genetic vulnerability, and residual confounding[5 19]. These mechanisms are not disease specific, and may also explain the link between depression and RA.

Although depression has been linked to disease activity in persons with RA, results of longitudinal studies on this relationship are inconsistent[12 13 20-25]. Most studies reported positive associations between higher scores of depression (or scores above a certain cut-off) on one hand, and smaller reductions in disease activity scores[12 13 20 22 25], a lower proportion of clinical remission[13 20] and a lower reduction of pain upon treatment[12 13 22 23 25], on the other hand. However, two studies could not confirm these positive associations[21 24]. Explanations may be the difference in follow-up times, i.e. 1-3.5 years for the positive studies and 5-10 years for the negative studies, different active treatment schedules which might have the biggest effect on the studies with shorter follow-up times, and the bidirectional association between depression and RA[6]. It is likely that the first symptoms and the diagnosis of RA lead to a depressive mood, whereas active treatment aims to reduce disease activity which in turn will lead to an improved mood. To exclude the reverse explanation that RA symptoms and treatment lead to changes in mood, studies on the influence of depressive mood on the onset of RA must be performed as early in the disease course as possible. It is important to know whether depression increases the risk of developing RA. If this is the case, treatment of depression could contribute to the prevention of arthritis.

Two studies have investigated the bilateral association between depressive mood and RA development in cohorts of persons without active RA or treatment for RA at the moment of inclusion. The first found a bidirectional relation between RA and depression in a Taiwanese health insurance database[6], and the second found that self-reported arthritis predicts the development of mood and anxiety disorders, no reverse association was found[26] These studies did not analyze the association between depressive mood and measures of disease activity or symptoms, and may have misclassified patients by using International Classification of Diseases Clinical Modification codes (ICD-9-CM) or self-report to identify patients with RA or depression[6 26]. Therefore, we studied the longitudinal association between depressive mood, daily stressors, avoidance coping, and social support as predictors, and the development of arthritis and clinical parameters (i.e. pain, morning stiffness, tender joint count (TJC), erythrocyte sedimentation rate (ESR)) as outcomes, in seropositive arthralgia patients at risk for developing RA. This is the first study that used physician-based diagnoses to explore these associations in a cohort with an increased risk for RA, but without overt inflammation (i.e. normal ESR values and no swollen joints). Depressive mood, daily stressors and clinical parameters were considered the primary predictors and outcomes, respectively.

PATIENTS AND METHODS

Study design and population

A 5-year follow-up study of a sample of 235 patients from the Reade seropositive arthralgia cohort (included between August 2004 and January 2011) was undertaken[27 28]. This cohort was formed to identify clinical and serological predictors for the development of arthritis (90% of whom fulfilled the 2010 ACR/EULAR criteria for RA)[28 29], and recruited persons with arthralgia and a positive anti-citrullinated protein antibodies (ACPA) and/or IgM-rheumatoid factor (hereafter RF) status. Demographic, clinical and laboratory measurements were performed every year until a complete follow-up of 5 years or arthritis development. Details on the study protocol have been published elsewhere[28]. All cohort participants were eligible for the present study and were sent questionnaires measuring depressive mood, daily stressors, avoidance coping and social support between March 2008 and January 2016. Questionnaires were only sent to patients without a diagnosis of arthritis, as it was explicitly the intention to investigate the relation of psychological parameters with the development of arthritis, and not with the period thereafter. Please note that baseline descriptives (**Table 1**) were taken at baseline of the present study. This means some patients turned out seronegative at baseline, while they were seropositive when entering the parent study. Patients included between April 2006 and January 2011 were sent questionnaires simultaneously with the clinical measurements at baseline, 3-year, 4-year, and 5-year follow-up (n=199). Patients included between April 2005 and April 2006 (n=22) were sent questionnaires after 3, 4 and 5 years, and patients included between August 2004 and March 2005 (n=14) were sent questionnaires after 4 and 5 years of follow-up. The study was conducted in compliance with the Declaration of Helsinki, and was approved by the medical ethics committee of the Slotervaart hospital and Reade.

All patients gave their written informed consent before entering the cohort study, and were additionally invited to complete the questionnaires.

MEASUREMENTS

Clinical endpoints
The first endpoint was the development of arthritis, as clinically diagnosed by a trained medical doctor based on a physical examination of 44 joints. The presence of arthritis in at least one joint was always confirmed by a senior rheumatologist(DvS). At the start of our study we did not know how many patients would progress to arthritis, and expected that changes in clinical parameters would precede the development of arthritis. Therefore, we selected a Visual Analogue Scale (VAS) for pain (range: 0-100), a VAS for morning stiffness (0-100), tender joint count 53 (TJC53) and the erythrocyte sedimentation rate (ESR) (reference <15 mm/hr for men, <20 mm/hr for women) as additional outcomes.

Questionnaire items
The primary independent variables were depressive mood, measured with the depression subscale of the Hospital Anxiety and Depression Scale (HADS)[30], and daily stressors, measured with the 49-item version of the Everyday Problem Checklist (EPCL)[31]. Secondary independent variables were avoidance coping, measured with the avoidance subscale of the Utrecht Coping List (UCL)[32], and social support, measured with the perceived support scale of the Impact of Rheumatic Diseases on General Health and Lifestyle (IRGL) questionnaire[33].

The Hospital Anxiety and Depression Scale (HADS)
The HADS depression subscale comprises 7 items that are answered on a 4-point scale. The sum score ranges from 0 to 21, with a higher score indicating a more depressive mood[30]. This sum score was used in analyses. In addition, we calculated cut-off scores to describe the degree of depressive mood in a more discrete way. These cut-off scores are based on findings of Zigmond and Snaith, who reported that a score of 8-10 is suggestive for the presence of a depressive disorder and a score ≥11 indicates probable presence ('caseness') of a depressive disorder[30 34]. Both the sum score and the cut-off scores are presented in **Table 1**. The HADS is widely used in clinical research, and has been shown to be reliable for use as a screening tool in different groups of Dutch adults aged 18-65 years (Cronbach's alpha: 0.77 to 0.86)[35].

The Everyday Problem Checklist (EPCL)
The EPCL comprises 49 items that describe irritating, annoying or disappointing events and situations, for which the patient has to indicate whether he or she had dealt with it in the past 4 weeks[31] .The sum score ranges from 0 to 49, with a higher score indicating more daily stressors. The reliability of the ECPL sum score has been shown to be satisfactory in Dutch adults[31].

The Utrecht Coping List (UCL)
The avoidance subscale of the UCL comprises 8 items that are answered on a 4-point scale[32]. The sum score ranges from 8 to 32, with a higher score indicating a more frequent use of avoidance coping. The UCL avoidance subscale has been shown to be sufficiently reliable in Dutch adults (Cronbach's alpha: 0.74 to 0.76)[36].

The perceived support scale of the Impact of Rheumatic Diseases on General Health and Lifestyle (IRGL) questionnaire
The perceived support subscale of the IRGL comprises 5 items that are answered on a 4-point scale[33]. The sum score ranges from 5 to 20, with a higher score indicating a higher perception of social support. The IRGL perceived support scale has been shown to be highly reliable in Dutch adults (Cronbach's alpha: 0.94)[37].

Statistical analyses
First, we explored changes in depressive mood, daily stressors, avoidance coping and social support between baseline and 5-year follow-up using descriptive statistics.

Second, we performed Cox regression analyses with time-dependent covariates to analyze the association between the independent variables depressive mood, daily stressors, avoidance coping and social support, and the development of arthritis. Hereby, the independent variables were time-updated to use all available questionnaire sum scores at the different time points. All independent variables were included as continuous variables in the Cox analyses. An additional analysis was done with depression as a dichotomous variable (HADS depression score ≥ 11) to facilitate interpretation (**Table 2**, model 1b).

Third, mixed models with a random intercept were used to evaluate the longitudinal associations between the independent variables depressive mood, daily stressors, avoidance coping and social support (measured on a continuous scale), and the outcome variables VAS pain, VAS morning stiffness, TJC53 and ESR[38]. In our design we took multiple measures per individual. That is, each individual completed multiple questionnaires at multiple times, and was clinically assessed at the same moments. Multiple responses from the same individual cannot be regarded as independent from each other, however linear mixed effects models correct for this independence. This implies that measured variables at all time points were included in the models. Separate analyses were performed for both the four independent variables and the four dependent variables. The random intercept accounts for the correlated nature of multiple measurements from the same individual. To distinguish between "between-subject and within-subject" effects, in addition, hybrid mixed models were performed[39].

Depending on the distribution of the outcome variable, we used different mixed models. Tobit mixed model analyses were performed for the two VAS scores, taking into account left censoring[40]. Negative binomial mixed models were used for TJC53 and linear mixed models for ESR. Before the analyses, ESR was log transformed, because of skewness to the right. We identified age, gender and symptom duration as potential confounders.

In step 2 of all analyses we adjusted for these variables.

Mixed models were computed using Stata version 14 (StataCorp. 2014, College Station, TX: StataCorp LP), and Cox regression analyses were performed with SPSS version 21 (IBM Corp, Armonk, NY, USA).

RESULTS

Study population

Of the 235 persons invited to participate in the study, 4 persons completed none of the questionnaires and were therefore excluded. Of the remaining 231 patients, 79 (34.2%) developed arthritis during the study, after a median of 12 (25^{th}-75^{th} percentiles 5-25) months. Post-arthritis no further questionnaires were sent to these patients. In all patients that should have completed the questionnaire at the different time points (taking into account "dropouts" due to the incident diagnosis of arthritis), 185 (80.1%), 118 (72.4%), 116 (71.2%) and 99 (62.7%) patients completed the baseline, 3-year, 4-year and 5-year follow-up questionnaire, respectively. The characteristics of the study population at the time of the first questionnaire are presented in **Table 1**.

Changes in depressive mood, daily stressors, avoidance coping and social support

Very small changes in HADS depression score (median [interquartile range (IQR)] at T0 = 4 (1-7), T3 = 3 (1-7), T4 = 3 (1-7), T5 = 3 (1-6)), EPCL daily stressors score (median [IQR] at T0 = 10 (6-16), T3 = 10 (6-15), T4 = 9 (5-14), T5 = 10 (5-15)), UCL avoidance score (mean ± standard deviation (SD) at T0 = 15.7 ± 3.3, T3 = 15.5 ± 3.0, T4 = 15.5 ± 3.1, T5 = 15.5 ± 3.1) and IRGL perceived support score (median [IQR] at T0 = 15 (12-18), T3 = 15 (13-18), T4 =15 (13-19), T5 = 15 (12-18) were observed between baseline and 5-year follow-up.

Table 1. Characteristics of the 231 arthralgia patients at the time of the first questionnaire

	Total (n=231)	**Patients who were diagnosed with arthritis durig the study (n=79)**	**Patients who were not diagnosed with arthritis (n=152)**
Characteristic	**Value**	**Value**	**Value**
Age (years), mean (SD)	49.6 (11.4)	47.6 (11.0)	50.6 (11.6)
Gender (female), no. (%)	179 (77.5)	62 (78.5)	117 (77.0)
Duration of symptoms (months), median (IQR)	17.1 (11.9 – 36.4)	17.1 (9.3 – 36.1)	17.1 (12.0 – 36.5)
IgM-RF and ACPA status			
IgM-RF positive, ACPA negative, no. (%)	73 (31.6)	8 (10.1)	65 (42.8)
ACPA positive, IgM-RF negative, no. (%)	97 (42.0)	40 (50.6)	57 (37.5)
IgM-RF and ACPA positive, no. (%)	50 (21.6)	31 (39.2)	19 (12.5)
IgM-RF and ACPA negative, no. (%)	11 (4.8)	0 (0)	11 (7.2)
ESR (mm/hr), median (IQR)	12.5 (6.0 – 20.0)	12.5 (4.0 – 21.3)	12.5 (7.0 – 20.0)

table continues

	Total (n=231)	Patients who were diagnosed with arthritis durig the study (n=79)	Patients who were not diagnosed with arthritis (n=152)
Characteristic	**Value**	**Value**	**Value**
VAS pain (range: 0-100), median (IQR)	26 (0 – 50)	33 (15 – 60)	23 (0 – 48)
VAS morning stiffness (range: 0-100), median (IQR)	14 (0 – 42)	26 (0 – 59)	0 (0 – 33)
Tender Joint Count 53 (range: 0-53), median (IQR)	0 (0 – 3)	1 (0 – 5)	0 (0 – 2)
HADS depression score (range: 0-21), median (IQR)	4 (1 – 7)	4 (2 – 8)	4 (1 – 7)
HADS score ≥8 (suggestive of the presence of depressed mood), no. (%)	54 (23.4)	20 (25.3)	34 (22.4)
HADS score ≥11 (probable presence of depression), no. (%)	24 (10.4)	12 (15.2)	12 (7.9)
Everyday Problem Checklist score (range: 0-49), median (IQR)	10 (6 – 16)	10 (6 – 17)	10 (6 – 15)
UCL avoidance score (range: 8-32), mean (SD)	15.7 (3.3)	15.4 (3.1)	15.8 (3.4)
IRGL perceived support score (range: 5-20), median (IQR)	15 (12 – 18)	15 (14 – 18)	15 (12 – 19)
Follow-up time (months), median (IQR)	33 (13 – 59)	12 (5 – 25)	48 (23 – 60)

ACPA = anti-citrullinated protein antibodies; ESR = Erythrocyte Sedimentation Rate, HADS = Hospital Anxiety and Depression Scale, IQR = Interquartile Range, IRGL = Impact of Rheumatic diseases on General Health and Lifestyle, RF = Rheumatoid Factor, SD = Standard Deviation, UCL = Utrecht Coping List, VAS = Visual Analogue Scale.

Development of arthritis

The Cox regression analyses revealed no statistically significant associations between depressive mood, daily stressors, avoidance coping or social support and the development of arthritis (hazard ratios [HR's] between 0.98 and 1.04, **Table 2**). Results did not change after adjustment for potential confounders.

Table 2. Cox regression of development of arthritis on depressive mood, daily stressors, avoidance coping and social support

		Development of arthritis		
Model	**Independent variables**	**HR**	**95% CI**	**p-value**
1a (n = 228)	Depressive mood (HADS depression score)	1.04	0.98, 1.09	0.208
1b (n = 228)	Depressive mood (HADS depression score ≥ 11)	1.82	0.96, 3.44	0.068
2 (n = 230)	Daily stressors (EPCL frequency score)	1.01	0.99, 1.04	0.352

table continues

		Development of arthritis		
Model	**Independent variables**	**HR**	**95% CI**	**p-value**
3 (n = 227)	Avoidance coping (UCL avoidance score)	0.98	0.91, 1.05	0.577
4 (n = 231)	Social support (IRGL perceived support score)	0.98	0.92, 1.04	0.438

The independent variables depressive mood, daily stressors, avoidance coping and social support were time-updated. In all models, adjustment for age, gender and symptom duration did not change the results (data not shown). EPCL = Everyday Problem Checklist; HADS = Hospital Anxiety and Depression Scale; HR = hazard ratio; IRGL = Impact of Rheumatic diseases on General Health and Lifestyle; UCL = Utrecht Coping List; 95% CI = 95% confidence interval.

Clinical parameters

Depressive mood and daily stressors.

Regular and hybrid tobit mixed models showed that the HADS depression score was statistically significantly associated with VAS pain (regression coefficient [B] 2.34, 95% confidence interval [95% CI] 1.59 to 3.08, $P < 0.001$) and VAS morning stiffness (B 4.09, 95% CI 1.18 to 7.00, $P = 0.006$) (**Table 3**). The regression coefficients of 2.34 and 4.09 have a combined between-subject (i.e. cross-sectional) and within-subject (i.e. longitudinal) interpretation, and can be interpreted as follows: a one-unit difference in the HADS depression score between two patients or a one-unit increase in the HADS depression score within one patient is associated with a difference or increase of 2.34 and 4.09 units in the VAS pain score and VAS morning stiffness score, respectively. Regular and hybrid negative binomial mixed models showed that the HADS depression score was statistically significantly associated with TJC53 (rate ratio [RR] 1.06, 95% CI 1.03 to 1.09, $P < 0.001$) (**Table 3**). Interpretation: if the HADS depression score is one unit higher in one patient compared to another patient or increases with one unit within one patient then the TJC53 will be 1.06 times higher. B's and RR's can be split into a between-subject (i.e. cross-sectional) and within-subject (i.e. longitudinal) effect, which are presented in **Table 3**. For VAS pain, the between-subject effect and the within-subject effect were both statistically significant, however the between-subject effect was stronger, indicating greater differences in pain between two patients with different HADS depression scores (cross-sectional) than changes in pain within patients with changing HADS scores (longitudinal). For VAS stiffness and TJC53, only the between-subject effect was significantly associated with HADS depression scores. HADS depression scores and ESR were not associated (Exp[B] 1.01, 95% CI 0.99 to 1.03, $P = 0.23$). The regression coefficient of log transformed ESR was back transformed to Exp(B), and can be interpreted like the RR as presented above. Daily stressors were only significantly associated with VAS pain (B 0.44, 95% CI 0.04 to 0.84, $P = 0.03$), attributed mainly by the cross-sectional differences in scores between patients. None of the results changed after correction for potential confounders (**Supplementary Table 1**).

Avoidance coping and social support. Higher social support was significantly associated with a lower VAS pain (B -1.97, 95% CI -2.77 to -1.17, $P < 0.001$), VAS morning stiffness (B -4.33, CI -7.40 to -1.28, $P = 0.005$) and TJC53 (RR 0.93, 95% CI 0.91 to 0.96, $P < 0.001$) score (**Table 3**). No statistically significant longitudinal associations were found between avoidance coping and disease activity measures.

Table 3. Longitudinal regression of clinical parameters on depressed mood, daily hassles, avoidance coping and social support

		VAS pain			VAS morning stiffness			Tender Joint Count 53			Erythrocyte Sedimentation Rate		
	Independent variables	**B**	**95% CI**	**obs**	**B**	**95% CI**	**obs**	**RR**	**95% CI**	**obs**	**Exp(B)**	**95% CI**	**obs**
1	Depressed mood (HADS depression score)	**2.34**	**1.59, 3.08**	886	**4.09**	**1.18, 7.00**	892	**1.06**	**1.03, 1.09**	894	1.01	0.99, 1.03	821
	Between-subject effect	**2.63**	**1.76, 3.50**		**4.62**	**1.34, 7.89**		**1.08**	**1.04, 1.12**		1.03	1.00, 1.06	
	Within- subject effect	**1.55**	**0.11, 2.98**		2.14	-4.14, 8.41		1.02	0.97, 1.08		1.00	0.98, 1.02	
2	Daily stressors (EPCL frequency score)	**0.44**	**0.04, 0.84**	892	0.31	-1.19, 1.80	898	1.01	0.99, 1.02	900	1.00	0.99, 1.01	827
	Between-subject effect	**0.59**	**0.11, 1.07**		-0.15	-1.89, 1.58		1.01	0.99, 1.03		1.01	0.99, 1.02	
	Within- subject effect	0.12	-0.59, 0.83		1.67	-1.32, 4.66		0.99	0.97, 1.02		1.00	0.98, 1.01	
3	Avoidance coping (UCL avoidance score)	0.47	-0.49, 1.42	883	-0.66	-4.22, 2.89	889	1.03	1.00, 1.07	891	0.99	0.97, 1.01	818
	Between-subject effect	1.03	-0.15, 2.21		-0.22	-4.38, 3.94		1.01	0.97, 1.07		1.02	0.98, 1.06	
	Within- subject effect	-0.58	-2.19, 1.02		-1.87	-8.74, 4.99		1.05	0.99, 1.12		0.98	0.96, 1.01	
4	Social support (IRGL perceived support)	**-1.97**	**-2.77, -1.17**	892	**-4.34**	**-7.40, -1.28**	898	**0.93**	**0.91, 0.96**	900	1.00	0.98, 1.02	826
	Between-subject effect	**-2.24**	**-3.23, -1.25**		**-3.65**	**-7.26, -0.05**		**0.93**	**0.89, 0.97**		1.00	0.96, 1.03	
	Within- subject effect	**-1.47**	**-2.82, -0.12**		**-6.11**	**-11.93, -0.30**		**0.94**	**0.90, 0.99**		1.00	0.98, 1.02	

B = regression coefficient; analyzed with Tobit mixed model analyses taking into account left censoring. RR = Rate Ratio; analyzed with negative binomial mixed models taking into account left censoring in a variable in which a score of zero really means zero. Exp(B) = Exponent (regression coefficient); analyzed with linear mixed models in which erythrocyte sedimentation rate (ESR) was log transformed (ln [ESR]), because of skewness to the right.

Hybrid mixed models were used to split the B's and RR's into a between-subject (i.e. cross-sectional) and within-subject (i.e. longitudinal) effect. The between-subject effect shows the difference in the outcome variable between two patients having a one-unit difference in the independent variable. The within-subject effect shows the increase in the outcome variable within one patient when the independent variable increases with one unit.

EPCL = Everyday Problem Checklist; HADS = Hospital Anxiety and Depression scale; IRGL = Impact of Rheumatic diseases on General Health and Lifestyle; Obs = number of observations of the outcome used in analysis; UCL = Utrecht Coping List; VAS = visual analogue scale; 95% CI = 95% confidence interval.

In **bold** statistically significant associations with a p-value < 0.05.

DISCUSSION

This is the first prospective study that investigated the association between depressive mood, daily stressors, avoidance coping, social support and progression towards arthritis or clinical parameters in patients at risk of developing RA. An effect on the development of arthritis and its timing could not be demonstrated. However, we did find a strong association between high depressive mood, low social support and several clinically important parameters, such as VAS pain, VAS morning stiffness and TJC53. No consistent associations were found between daily stressors, avoidance coping and any of the clinical parameters.

One can speculate about the mechanism by which high depressive mood is associated with higher clinical parameter scores in these patients: is it purely psychological or also biological? As a person's psychological state plays a key role in the experience and expression of (joint) symptoms and vice versa, a psychological mechanism seems likely. Depressive symptoms and stress are believed to influence clinical parameters via negative perceptions of symptoms and non-adherence to medical recommendations[5 9]. However, it is also possible that depression leads partly to a higher presence of clinical parameters through biological mechanisms, which may or may not be induced by behavior. Possible biological mechanisms are dysregulation of the immune system[5 16 41], HPA-axis or metabolism[16 41 42], which may be induced by a shared genetic vulnerability leading to both depression and RA[5]. A combined biological and behavioral mechanism are unhealthy lifestyle behaviors, such as smoking, physical inactivity and unhealthy diet, which are risk factors for both diseases, but may also be caused by depression[43-46]. Our results provide most support for a psychological mechanism, because depressive mood was mainly associated with subjectively reported clinical parameters, although we cannot rule out that the mechanism that connects depressive mood with clinical parameters is also partly biological. In our study population of arthralgia patients without active clinical disease, we had a limited set of biological disease activity measures available.

The association of low social support with an increase in disease activity is in line with results of a 5-year follow-up study in 78 persons with early RA[14]. Social support may influence clinical parameters via negative disease related cognitions, low self-efficacy, unfavorable coping, and unhealthy behaviors[14].

Besides discussing the mechanism by which depressive mood and low social support are associated with clinical parameters, one can also debate on the clinical implication of this finding. A recent study showed that the presence of depression reduced the success percentage of biologic treatment in patients with RA[47]. Probably because pain and perceived health are taken into account in disease activity scores such as the DAS28, and the sense of wellbeing is disrupted in patients with a depressive mood. This might indicate that early detection and treatment of depressive mood may benefit future treatment in arthralgia patients for whom interventions to prevent the development of RA are not available yet[48]. The potentially important influence of social support on the course of clinical parameters

is something that physicians also should be aware of. However, it may even be true that higher pain and fatigue scores are more a consequence of depression than of arthritis.

In analyses testing causal explanations one should not adjust for variables in the causal pathway. Therefore, in the analysis between 1 of the 4 questionnaire variables (i.e. depressive mood, daily stressors, avoidance coping and social support) and the outcome, we did not adjust for the other 3 questionnaire variables. However, to be on the safe side, we examined what happens when all questionnaire variables are included in the analyses (**Supplementary Table 2**). The results of these analyses show that even if we adjust for variables that are potentially on the causal path, the results are almost the same.

Our study may be criticized for the fact that, although our study population concerns a seropositive arthralgia cohort without active RA or treatment, it is becoming increasingly clear that patients already experience a high burden of symptoms that adversely affect daily functioning, and in turn may affect their mood[49 50]. As a result, we cannot completely rule out that early symptoms have had influence on subsequent depressive mood. Secondly, some patients entered the present study later than entering the prospective cohort of auto-antibody positive arthralgia. This might have introduced selection bias, as patients with arthritis development had already been censored. However, in daily practice when a particular arthralgia patient presents to the rheumatologist we can also only tell in retrospect the stage of preclinical RA he or she was at. Thirdly, we did not adjust for educational level in the analyses. Although a low level of education or socioeconomic status does not appear to be a strong risk factor for the development of arthritis, it is associated with depression, daily stressors, coping behavior and social support. Therefore, it is possible that educational level has slightly biased the association found between the psychological variables and clinical parameters.

In conclusion, our findings highlight that an effect of psychological parameters on arthritis development could not be demonstrated in seropositive arthralgia patients. However, a strong longitudinal association was found between high depressive mood, low social support and clinical parameters. For clinicians it is important to be aware that, already in patients at risk of developing arthritis, depressive symptoms and low social support may increase musculoskeletal symptoms.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Adjusted longitudinal regression of clinical parameters on depressed mood, daily stressors, avoidance coping and social support

		VAS pain			VAS morning stiffness			Tender Joint Count 53			Erythrocyte Sedimentation Rate		
	Independent variables	**B**	**95% CI**	**obs**	**B**	**95% CI**	**obs**	**RR**	**95% CI**	**obs**	**Exp (B)**	**95% CI**	**obs**
1	Depressed mood (HADS depression score)	**2.3**	**1.6, 3.1**	874	**4.2**	**1.3, 7.1**	880	**1.06**	**1.03, 1.09**	882	1.01	0.99, 1.03	810
	Between-subject effect	**2.6**	**1.8, 3.5**		**4.8**	**1.4, 8.1**		**1.08**	**1.04, 1.12**		1.02	0.99, 1.05	
	Within- subject effect	**1.5**	**0.1, 3.0**		2.2	-4.2, 8.5		1.03	0.98, 1.08		1.00	0.98, 1.03	
2	Daily stressors (EPCL frequency score)	**0.5**	**0.1, 0.9**	880	0.5	-1.1, 2.0	886	1.01	0.99, 1.02	888	1.00	0.99, 1.01	816
	Between-subject effect	**0.7**	**0.2, 1.2**		0.04	-1.7, 1.8		1.01	0.99, 1.04		1.01	1.00, 1.03	
	Within- subject effect	0.1	-0.6, 0.8		1.6	-1.4, 4.6		0.99	0.97, 1.02		1.00	0.98, 1.01	
3	Avoidance coping (UCL avoidance score)	0.4	-0.6, 1.4	871	-0.5	-4.1, 3.2	877	1.03	0.99, 1.07	879	0.99	0.97, 1.01	807
	Between-subject effect	0.98	-0.2, 2.2		0.2	-4.2, 4.5		1.01	0.97, 1.06		1.00	0.97, 1.04	
	Within- subject effect	-0.6	-2.2, 1.0		-2.0	-8.9, 4.9		1.05	0.99, 1.12		0.98	0.96, 1.01	
4	Social support (IRGL perceived support score)	**-2.0**	**-2.8, -1.2**	880	**-4.4**	**-7.5, -1.3**	886	**0.93**	**0.90, 0.96**	888	1.00	0.98, 1.02	815
	Between-subject effect	**-2.2**	**-3.2, -1.2**		**-3.7**	**-7.4, -0.05**		**0.93**	**0.89, 0.96**		0.99	0.96, 1.02	
	Within- subject effect	**-1.5**	**-2.8, -0.2**		**-6.1**	**-12.0, -0.3**		**0.94**	**0.90, 0.99**		1.01	0.98, 1.03	

B = regression coefficient adjusted for age, gender and symptom duration; analyzed with Tobit mixed model analyses taking into account left censoring. RR = Rate Ratio adjusted for age, gender and symptom duration; analyzed with negative binomial mixed models taking into account left censoring in a variable in which a score of zero really means zero. Exp(B) = Exponent (regression coefficient) adjusted for age, gender and symptom duration; analyzed with linear mixed models in which erythrocyte sedimentation rate (ESR) was log transformed (ln [ESR]), because of skewness to the right.

Hybrid mixed models were used to split the B's and RR's into a between-subject (i.e. cross-sectional) and within-subject (i.e. longitudinal) effect.

The between-subject effect shows the difference in the outcome variable between two patients having a one-unit difference in the independent variable. The within-subject effect shows the increase in the outcome variable within one patient when the independent variable increases with one unit.

EPCL = Everyday Problem Checklist; HADS = Hospital Anxiety and Depression scale; IRGL = Impact of Rheumatic diseases on General Health and Lifestyle; Obs = number of observations of the outcome used in analysis; UCL = Utrecht Coping List; VAS = visual analogue scale; 95% CI = 95% confidence interval.

In **bold** statistically significant associations with a p-value < 0.05.

Supplementary Table 2. Longitudinal regression of clinical parameters on depressed mood, daily stressors, avoidance coping and social support: univariate (in black) and multivariate (in gray) results

		VAS pain			VAS morning stiffness			Tender Joint Count 53			Erythrocyte Sedimentation Rate		
	Independent variables	**B**	**95% CI**	**obs**	**B**	**95% CI**	**obs**	**RR**	**95% CI**	**obs**	**Exp(B)**	**95% CI**	**obs**
1	Depressed mood (HADS depression score)	**2.34**	**1.59, 3.08**	886	**4.09**	**1.18, 7.00**	892	**1.06**	**1.03, 1.09**	894	1.01	0.99, 1.03	821
	Between-subject effect	**2.63**	**1.76, 3.50**		**4.62**	**1.34, 7.89**		**1.08**	**1.04, 1.12**		1.03	1.00, 1.06	
	Within- subject effect	**1.55**	**0.11, 2.98**		2.14	-4.14, 8.41		1.02	0.97, 1.08		1.00	0.98, 1.02	
	Depressed mood adj	**1.85**	**0.96, 2.73**	859	**3.65**	**0.05, 7.25**	865	**1.04**	**1.01, 1.08**	867	1.01	0.99, 1.03	797
	Between-subject effect	**2.13**	**1.08, 3.18**		**4.35**	**0.25, 8.45**		**1.06**	**1.02, 1.11**		1.03	1.00, 1.06	
	Within- subject effect	1.28	-0.17, 2.72		1.73	-4.74, 8.21		1.01	0.96, 1.06		1.00	0.98, 1.03	
2	Daily stressors (EPCL frequency score)	**0.44**	**0.04, 0.84**	892	0.31	-1.19, 1.80	898	1.01	0.99, 1.02	900	1.00	0.99, 1.01	827
	Between-subject effect	**0.59**	**0.11, 1.07**		-0.15	-1.89, 1.58		1.01	0.99, 1.03		1.01	0.99, 1.02	
	Within- subject effect	0.12	-0.59, 0.83		1.67	-1.32, 4.66		0.99	0.97, 1.02		1.00	0.98, 1.01	
	Daily hassles adj	0.20	-0.21, 0.61	859	-0.31	-1.95, 1.33	865	1.00	0.98, 1.01	867	1.00	0.99, 1.01	797
	Between-subject effect	0.21	-0.29, 0.70		1.02	-2.93, 0.89		1.00	0.98, 1.02		1.01	0.99, 1.02	
	Within- subject effect	0.19	0.52, 0.90		1.58	-1.52, 4.68		1.00	0.97, 1.02		1.00	0.99, 1.01	
3	Avoidance coping (UCL avoidance score)	0.47	-0.49, 1.42	883	-0.66	-4.22, 2.89	889	1.03	1.00, 1.07	891	0.99	0.97, 1.01	818
	Between-subject effect	1.03	-0.15, 2.21		-0.22	-4.38, 3.94		1.01	0.97, 1.07		1.02	0.98, 1.06	

table continues

		VAS pain			VAS morning stiffness			Tender Joint Count 53			Erythrocyte Sedimentation Rate		
	Independent variables	**B**	**95% CI**	**obs**	**B**	**95% CI**	**obs**	**RR**	**95% CI**	**obs**	**Exp(B)**	**95% CI**	**obs**
	Within- subject effect	-0.58	-2.19, 1.02		-1.87	-8.74, 4.99		1.05	0.99, 1.12		0.98	0.96, 1.01	
	Avoidance coping adj	-0.68	-1.64, 0.28	859	-2.96	-6.87, 0.94	865	1.00	0.96, 1.04	867	0.99	0.97, 1.01	797
	Between-subject effect	-0.59	-1.79, 0.61		-3.21	-7.88, 1.45		0.96	0.91, 1.01		1.00	0.96, 1.04	
	Within- subject effect	-0.84	-2.42, 0.74		-2.40	-9.42, 4.63		1.05	0.99, 1.11		0.98	0.96, 1.01	
4	Social support (IRGL perceived support)	**-1.97**	**-2.77, -1.17**	892	**-4.34**	**-7.40, -1.28**	898	**0.93**	**0.91, 0.96**	900	1.00	0.98, 1.02	826
	Between-subject effect	**-2.24**	**-3.23, -1.25**		**-3.65**	**-7.26, -0.05**		**0.93**	**0.89, 0.97**		1.00	0.96, 1.03	
	Within- subject effect	**-1.47**	**-2.82, -0.12**		**-6.11**	**-11.93, -0.30**		**0.94**	**0.90, 0.99**		1.00	0.98, 1.02	
	Social support adj	**-1.16**	**-2.06, -0.27**	859	-3.38	-7.05, 0.28	865	**0.95**	**0.91, 0.98**	867	1.01	0.99, 1.02	797
	Between-subject effect	**-1.18**	**-2.32, -0.04**		-2.65	-7.10, 1.79		**0.95**	**0.90, 0.99**		1.00	0.97, 1.04	
	Within- subject effect	-1.14	-2.51, 0.23		-4.77	-10.81, 1.27		0.95	0.90, 1.00		1.01	0.98, 1.03	

B = regression coefficient; analyzed with Tobit mixed model analyses taking into account left censoring. RR = Rate Ratio; analyzed with negative binomial mixed models taking into account left censoring in a variable in which a score of zero really means zero. Exp(B) = Exponent (regression coefficient); analyzed with linear mixed models in which erythrocyte sedimentation rate (ESR) was log transformed (ln [ESR]), because of skewness to the right.

Hybrid mixed models were used to split the B's and RR's into a between-subject (i.e. cross-sectional) and within-subject (i.e. longitudinal) effect. The between-subject effect shows the difference in the outcome variable between two patients having a one-unit difference in the independent variable. The within-subject effect shows the increase in the outcome variable within one patient when the independent variable increases with one unit.

EPCL = Everyday Problem Checklist; HADS = Hospital Anxiety and Depression scale; IRGL = Impact of Rheumatic diseases on General Health and Lifestyle; Obs = number of observations of the outcome used in analysis; UCL = Utrecht Coping List; VAS = visual analogue scale; 95% CI = 95% confidence interval.

In **bold** statistically significant associations with a p-value < 0.05. In red adjusted for age, gender, duration of symptoms, HADS depression score, EPCL frequency score, UCL avoidance score, IRGL perceived support score.

CHAPTER 5

Initial validation and results of the Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire, a EULAR project

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ABSTRACT

Objectives: To describe the development and assess the psychometric properties of the novel "Symptoms in Persons At Risk of Rheumatoid Arthritis" (SPARRA) questionnaire in individuals at risk of rheumatoid arthritis (RA) and to quantify their symptoms.

Methods: The questionnaire items were derived from a qualitative study in seropositive arthralgia patients. The questionnaire was administered to 219 individuals at risk of RA on the basis of symptoms or autoantibody positivity: 74% rheumatoid factor and/or anti-citrullinated protein antibodies positive, 26% seronegative. Validity, reliability and responsiveness were assessed. Eighteen first degree relatives (FDR) of patients with RA were used for comparison.

Results: Face and content validity were high. The test-retest showed good agreement and reliability (one week and 6 months). Overall, construct validity was low to moderate, with higher values for concurrent validity, suggesting that some questions reflect symptom content not captured with regular VAS pain/well-being. Responsiveness was low (small subgroup). Finally, the burden of symptoms in both seronegative and seropositive at risk individuals was high, with pain, stiffness and fatigue being the most common ones with a major impact on daily functioning. The FDR cohort (mostly healthy individuals) showed a lower burden of symptoms, however the distribution of symptoms was similar.

Conclusions: The SPARRA questionnaire has good psychometric properties and can add information to currently available clinical measures in individuals at risk of RA. The studied group had a high burden and impact of symptoms. Future studies should evaluate whether SPARRA data can improve the prediction of RA in at risk individuals.

KEY MESSAGES

What is already known about this subject?

- A wide range of symptoms can be present in individuals at risk of rheumatoid arthritis, including extra-articular symptoms
- These symptoms can be severe and disabling

What does this study add?

- This study used data from qualitative focus interviews to quantify symptoms in individuals at risk of rheumatoid arthritis
- The "Symptoms in Persons At Risk of Rheumatoid Arthritis" (SPARRA) questionnaire provides information on location, timing and severity of these symptoms in a large international sample of individuals at risk of developing rheumatoid arthritis

How might this impact on clinical practice?

- The SPARRA questionnaire can be used to document symptoms in studies of persons at risk of rheumatoid arthritis

INTRODUCTION

A range of symptoms can be present in individuals at risk of rheumatoid arthritis (RA). These individuals are usually defined based on either autoantibody positivity or symptoms. In seropositive at risk persons, symptoms usually occur later than seropositivity[1 2]. However, information on location, timing and severity of symptoms is still largely lacking[3].

Symptoms such as joint pain, swelling and morning stiffness represent key elements in the diagnosis of RA. Clinicians have tried to use these and other symptoms to identify those at risk of RA before they fulfill classification criteria for this condition[4]. A European League Against Rheumatism (EULAR) taskforce recently outlined symptoms that were deemed most relevant in differentiating those at risk of developing RA (also known as "clinically suspect arthralgia" (CSA)[5]) from other patients with non-specific joint symptoms[6]. The criteria set for CSA was based on expert opinion and has shown value in predicting arthritis[7]. Qualitative research in individuals at risk of RA provided a different starting point to evaluate symptoms using the experience of the affected persons to understand the range of their symptomatology. With this approach multiple focus group interviews were performed in seropositive arthralgia patients[8-10]. Besides symptoms originating from the joints, additional extra-articular themes emerged such as fatigue, distress and loss of motor control, with a reported major impact on daily functioning. The presumed impact of such early symptoms is underscored by increased sick leave and medical ambulatory costs long before diagnosis of RA[11 12].

The "Symptoms in Persons At Risk of Rheumatoid Arthritis" (SPARRA) questionnaire was developed based on data from our previous qualitative study[9]. The aim of the present study was to describe the developmental process and test the psychometric properties of the SPARRA questionnaire in an international convenience sample of individuals at risk of RA (both autoantibody positive and negative), and to quantify and describe their symptoms based on this questionnaire.

PATIENTS AND METHODS

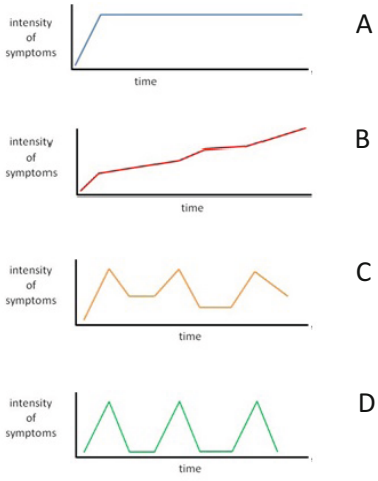
The development of the SPARRA questionnaire

The content of the SPARRA questionnaire is based on focus group interviews in 15 seropositive arthralgia patients and 11 early RA patients with whom initial symptoms prior to the diagnosis of RA were explored[9]. These semi-structured interviews were conducted to explore perceptions of symptoms, impact of symptoms and reactions to symptoms and continued until thematic saturation was reached. The content of the questionnaire was also informed by a previous review of the literature related to the earliest symptoms of RA[4] and prior research describing domains that were deemed important in predictive algorithms in these at-risk individuals[13 14]. The emerging themes were grouped and the most noteworthy and frequently occurring categories were selected. Feedback from the study team (two rheumatologists, one epidemiologist, one expert on psychological testing and two research patient partners) was used to discuss which symptoms to be

captured within the questionnaire, discuss realistic timeframes for symptom duration and the number/format of answer categories for severity and impact of the symptoms, taking into account reported variation in each of the domains by individuals from the focus group interviews. Afterwards, four rheumatologists from different countries, working in the field of the at risk phase of RA but who were not otherwise involved in the project, gave their feedback on the questionnaire. The final questionnaire included 13 symptoms, for which severity and impact were described from none to severe and no to high, respectively. Additional questions were aimed at capturing location and pattern of joint pain (if present) and the presence of morning stiffness. Recently, data on symptoms in the at risk phase of RA appeared in literature from two other cohorts and one review. These studies contain items on functional limitations, such as difficulty making a fist, which are possibly additive to the SPARRA questionnaire which only contains the following symptoms on function: "weakness or loss of motor control" and "impact of symptoms on daily functioning"[15-17]. The questionnaire's design and content was thereafter discussed with patient research partners from both Amsterdam and Birmingham to assess face validity which led to only minor comments and small modifications.

Subsequently the questionnaire was translated from English into Dutch, Swedish, German and French by at least one native speaker. These native speakers were part of the study teams at the different centers, had knowledge of research in individuals at risk of developing RA, but were not part of the study team that performed the focus interviews leading to the questionnaire development. Thereafter, another researcher from that study team translated it back to English, blinded for the original wording of the items, to complete the formal forward-backward approach as presented by the World Health Organization (WHO; steps 1 and 2, except for the fact that back-translation was not performed by a whole expert panel)[18]. All inconsistencies were resolved in collaboration with a member of the original focus group interview team (LvT), by referring to the original wording in the focus groups. Cross-cultural adaptations were made taking into account cultural aspects of presenting joint symptoms within the different countries[19]. As a preliminary pilot-test, the Dutch pre-final version of the questionnaire was administered to 30 seropositive individuals with arthralgia from the Netherlands, which did not change the questionnaire (WHO steps 3 and 4, no cognitive interviewing was performed)[18]. To preempt any missing symptoms individuals had the possibility of adding up to two additional symptoms that they thought were relevant. See Table 1 for an outline of the questionnaire (complete questionnaire and translated versions presented as Supplementary Figures 1-5).

Table 1: Outline of the Symptoms in Persons At Risk for Rheumatoid Arthritis (SPARRA) questionnaire

Per symptom [see right], the following questions are asked: a) Over the past month how many days of the month have you had [symptom]? b) Over the past month how much [symptom] have you had? c) What impact has this [symptom] had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)? d) Where did you feel the [symptoms 1 to 9] Answer categories: a) 0 days, 1 to 5 days, 6 to 16 days, 16 to 30 days b) None, mild, moderate, severe c) No impact, small impact, moderate impact, large impact d) Hand (one or both), arm (one or both), foot (one or both), leg (one or both)	1 Joint pain [symptoms] 2 Joint swelling 3 Joint stiffness 4 Burning sensations 5 Tingling sensations 6 Numbness 7 Changes in skin colour over joints 8 Muscle cramps 9 Weakness or loss of strength 10 Fatigue 11 Emotional distress 12 Concentration difficulties 13 Sleep problems
Additional questions: - Description of joint pain (burning, sharp/stabbing, aching, other) - Movement of joint pain (no, arms to legs, legs to arms, one side to the other) - Presence of morning stiffness (no, <1 hour, 1 to 2 hours, all morning) Rate the average joint pain over the last month in different body areas: - Answer categories: no pain, mild moderate, severe - Fingers (left/right), wrist (left/right), elbow (left/right), shoulder (left/right), hip (left/right), knee (left/right), ankle (left/right), toes (left/right), neck, back What is the pattern of symptom development since the time they first began (see patterns on the right; patients could also draw a pattern themselves)	

Note: full questionnaire (with translations) added as Supplementary Material

Study participants

To test the psychometric properties of the SPARRA questionnaire, individuals at risk of developing RA defined as individuals with RA-specific autoantibodies (anti-citrullinated protein antibodies (ACPA) and/or rheumatoid factor (RF)) or the presence of relevant symptoms (i.e. individuals with CSA based on clinical expertise in the different centers with or without RA specific antibodies) were selected from four European centres: Reade, Amsterdam (N=125) (further called the Netherlands), Sandwell and West Birmingham

Hospitals and the University Hospitals Birmingham (N=69) (United Kingdom), Karolinska University Hospital (N=15) (Sweden), and the Medical University of Vienna (N=10) (Austria). We have used an international sample of patients at risk for RA, mainly containing consecutive cohort patients (Netherlands, UK, Sweden, in total 88%) and complemented by a convenience sample from Austria and Switzerland. Please note that in Austria patients were recruited from their "pre-arthritis" cohort, and by additionally searching through their clinical database for ACPA and/or RF positive patients without a diagnosis of arthritis. In Switzerland individuals could complete a SPARRA questionnaire at any time when they visited for a yearly cohort follow-up. All cohorts were set up to characterize individuals at risk of developing RA and included individuals without prior arthritis (see Supplementary Table 1)[20 21]. Arthritis was assessed clinically (by a rheumatologist in all cohorts) by presence of at least one swollen joint: no confirmation by ultrasonography or MRI was used. A cohort of 18 first degree relatives (FDR) of patients with RA from the University Hospital of Geneva (Switzerland) was used as comparison, since they also represent a group of individuals at risk of developing RA which the SPARRA questionnaire is aimed at. This cohort was dealt with separately, since the individuals were recruited based on the fact that they were FDR and not because of symptoms or antibodies, and thus included mostly healthy individuals with an increased risk of RA. Individuals were included between November 2014 and December 2016.

Study procedures

At baseline, individuals completed the SPARRA questionnaire and had clinical data collected, including antibody status, total painful (tender joint count 44) and swollen joints (swollen joint count 44), family history, symptom duration, smoking status, Visual Analogue Scales (VAS, ranged 0-100) for pain, patient global assessment and fatigue. Detailed data on comorbidities and medication has not been consistently assessed across the cohorts for the present study. A subgroup of individuals had a follow-up measurement (test-retest) after one week and six months. Questionnaires completed at the time of or after clinical arthritis development (confirmed by a rheumatologist) were discarded for the current analysis. The study was approved by relevant Ethics Committees and all individuals gave written informed consent.

Psychometric properties

Content validity: Relevant medical articles on symptoms in the at risk phase of RA were compared to the questionnaire items to see if all relevant facets of the construct had been captured.

Construct validity: We performed correlations between baseline questionnaire items and clinical parameters that were deemed to be associated based on expert opinion (seven representatives from all centres). We divided these into items with a very close match (concurrent validity, for example the item fatigue compared to VAS fatigue) and items with less close a match (construct validity). Individuals had to complete the questionnaire within two weeks of clinical measurements.

Agreement and reliability: Test-retest analyses were performed in a subgroup of individuals that completed a second test within 7-14 days and/or after six months. The retest questionnaire was only sent after return of the first questionnaire. The analyses were performed in questions not containing time elements. Also, we described the scale reliability at baseline, i.e. looking at how closely related the items in the questionnaire are as a group.

Responsiveness: This can only be tested in individuals with expected change in their disease status, in our case VAS scores over a time period of 6 months. No formal clinically relevant VAS score changes have been described in individuals at risk of developing RA. We chose a change of 11 mm as sometimes used in an adult rheumatology setting[22], and we measured a second arbitrary cut-off of 25 mm, since the questionnaire items are on a 4-point scale. We only analyzed questionnaire items that were concurrent with the VAS scores. The questionnaire had to be completed within 2 weeks from the clinical measurements.

Statistical analyses

Construct validity: Correlations were calculated using the Spearman's rank correlation coefficient. Statistical significance was set as a p-value less than 0.05. The VAS scores were used as continuous data. Interpretation: 0-0.30 small, 0.3-0.50 medium, 0.50-1 large[23].

Test-retest agreement and reliability: The percentage of agreement in the questionnaire items were given for questions on symptom severity and impact, and the Cohen's weighted kappa (reliability) was measured[24]. Interpretation: <0 no, 0-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial and 0.81-1 almost perfect agreement[25 26]. Scale reliability was described with the Cronbach's alpha.

Responsiveness: The Wilcoxon signed-rank test was used to analyse significant differences between questionnaire items over the six month period, taking $p<0.05$ as significance level.

Handling missing data: All questions in the questionnaire follow the same pattern (Table 1). If sub questions "a" (duration of the symptom over the past month) were missing and "b-d" were also missing then "a" was set as zero days. Equally, if "a" was set as zero days, then "b-d" were set as none, no impact and not filled in respectively. Instead, if "b-d" were filled out while "a" was missing, then we assumed the worst case scenario and set "a" as 16-30 days.

All analyses were performed with SPSS version 21 (IBM Corp, Armonk, NY, USA), except for the Cohen's weighted kappa's which were computed using Stata version 13.1 (StataCorp. 2013, College Station, TX: StataCorp LP).

Frequency and impact of symptoms in the SPARRA questionnaire

Finally, we analysed data from individuals who were ACPA positive (with or without RF), only RF positive and those included in the cohort due to specific symptoms (seronegative CSA). Percentages of symptom duration in the last month (dichotomized to 0-15 days and

16 or more days), severity (none/mild versus moderate/severe) and impact (no/small versus moderate/large) were given for these groups, and information on joint pain location and patterns was described.

RESULTS

Study population

Two hundred nineteen individuals completed the SPARRA questionnaire in 4 European centres, with 18 FDR of patients with RA as comparison (Supplementary Table 2). Half of the individuals (excluding the FDR) were ACPA positive (with or without RF), 24% were only RF positive and 26% were seronegative with CSA (Table 2). The mean age of these study participants was 49 years (SD 13.2) and the median duration of symptoms was 20 months (25^{th}-75^{th} percentile 8-56).

Table 2: Baseline characteristics (N=219)

Variable	ACPA positive individuals[1] (N=109)	RF positive individuals[1] (N=53)	Seronegative individuals with CSA (N=57)	FDRs of patients with RA[2] (N=18)
Age[3]	49 (12.9)	54 (13.2)	45 (12.6)	57 (9.5)
Females (%)	72	64	72	89
Symptom duration (months)[4]	23 (10-52)	30 (12-60)	11 (4-39)	22 (7-51)
Tender Joint Count (44 joints)	0 (0-2)	0 (0-2)	2 (0-7)	1 (0-3)
VAS pain (mm)[4]	18 (2-56)	27 (3-47)	56 (34-71)	ND
VAS patient global assessment (mm)[4]	28 (3-56)	22 (0-49)	48 (25-69)	ND
VAS fatigue (mm)[4]	50 (9-80)	33 (6-59)	65 (40-82)	ND
Current smoking (%)	24	15	25	17
FDR with RA (%)[4]	29	21	28	100

[1] ACPA positive individuals: with or without RF positivity, RF positive individuals: only RF positive
[2] FDRs of patients from Switzerland with RA were used as comparison cohort
[3] Mean (standard deviation; SD), all other continuous variables mentioned as median (25^{th}-75^{th} percentile)
[4] Missing values; 2% for VAS global and family history, 3% for VAS pain, 4% for VAS fatigue, 6% for symptom duration, one individual for RF (marked as ACPA positive only now)

Netherlands:	ACPA + N=71	RF+ N=40	seronegative N=14
United Kingdom:	ACPA + N=21	RF+ N=8	seronegative N=40
Sweden:	ACPA + N=15	RF+ N=0	seronegative N=0
Austria:	ACPA + N=2	RF+ N=5	seronegative N=3
Switzerland:	ACPA + N=6	RF+ N=1	seronegative N=11

Abbreviations: ACPA: anti-citrullinated protein antibodies; RF: rheumatoid factor; CSA: clinically suspect arthralgia; FDR: first degree relatives; VAS: visual analogue scale; FDR: first degree relative; RA: rheumatoid arthritis; ND: not done.

Content validity

The items in the questionnaire represent symptoms that are important for individuals at risk of RA[13 14 27-29]. Literature search did not identify any additional symptoms describing the at risk phase, except for self-reported functional limitation as part of a tool to detect early inflammatory arthritis[30] and difficulty in making a fist at physical examination in individuals with CSA[6]. In addition to the seropositive individuals, we also selected seronegative individuals with CSA and FDRs to achieve a good representation of the at risk population. To make sure that no key symptoms were omitted, in addition to the 13 predefined symptoms, individuals had the option of adding two symptoms. Forty-three out of the 219 individuals used this option (of whom 14 reported two options). Themes (reported at least twice) were: pain/inflammation around tendons or myalgia (N=7), pain only while using the joint (N=4), dry eyes (N=4), functional limitations (N=2), itching skin spots (N=2) and swelling in the groin or legs (N=2). However, many of them did not describe new symptoms (N=16 alternative diagnosis that they felt explained their symptoms such as osteoarthritis or hernia, N=5 explanatory description, N=2 unclear).

Construct validity (relation to clinical parameters)

Analyses were performed in 208 individuals, since 11 did not have clinical data collected within two weeks from the baseline questionnaire. Overall, the correlation between questionnaire items and clinical parameters (VAS pain, VAS global assessment and VAS fatigue) was medium to large with Spearman coefficients ranging from 0.38 to 0.63 (Supplementary Table 3, all statistically significant). Correlations were higher when strictly looking at concurrent validity (0.58 to 0.63). The percentage of missing values in the questionnaire items was 1% (15 missing questions were set on the worst case scenario, 91 were set on no symptoms) and in the clinical parameters 3%.

Test-retest reliability and agreement, and scale reliability

Analyses after one week were performed in 51 individuals (20 ACPA+, 26 RF+, 5 seronegative) and after six months in 90 individuals (37 ACPA+, 30 RF+, 23 seronegative). The median time difference in the latter 90 individuals between the questionnaires was 6 months (25^{th}-75^{th} percentiles: 5-10). Thirty-eight individuals had a retest after both one week and six months. Overall, the test-retest agreement for the questions was good to excellent (88-98%) after one week, and good reliability with Cohen's weighted kappa's between 0.60 and 0.90 was found (Table 3). After six months the agreement was 73-91%, with lower overall kappa's between 0.09 and 0.62. <1% of the data was missing (14 missing questions were set on the worst case scenario, 30 were set on no symptoms). Subgroup analysis was not feasible due to low numbers. The Cronbach's alpha for all items on duration, severity and impact were 0.859, 0.874 and 0.908 respectively (0.958 if all items were combined).

Table 3: Test-retest analysis of the SPARRA questionnaire (N=51 after one week, N=90 after six months)

Items	% agreement[1]		Weighted kappa		Percentage with the symptom[2]	
	1 week	*6 months*	*1 week*	*6 months*	*1 week*	*6 months*
Joint pain: severity[3] / impact[4]	84 / 92	77/77	0.60 / 0.78	0.37/0.41	75	80
Joint swelling: severity / impact	95 / 94	83/83	0.80 / 0.77	0.42/0.44	28	37
Joint stiffness: severity / impact	88 / 93	79/81	0.71 / 0.78	0.47/0.45	69	74
Burning sensations: severity / impact	90 / 92	81/83	0.64 / 0.61	0.43/0.42	20	33
Tingling sensations: severity / impact	90 / 93	85/84	0.62 / 0.64	0.53/0.35	41	40
Numbness: severity / impact	90 / 94	83/85	0.53 / 0.67	0.27/0.27	26	27
Changes in skin colour: severity / impact	96 / 98	83/91	0.67 / 0.71	0.09/0.20	20	20
Muscle cramps: severity / impact	92 / 95	84/87	0.63 / 0.71	0.43/0.39	28	36
Weakness or loss of strength: severity / impact	92 / 93	78/76	0.81 / 0.81	0.43/0.34	57	56
Fatigue: severity / impact	93 / 92	80/79	0.81 / 0.82	0.52/0.50	69	63
Emotional distress: severity / impact	93 / 95	77/80	0.78 / 0.83	0.37/0.41	47	39
Concentration difficulties: severity / impact	93 / 94	86/87	0.76 / 0.78	0.58/0.58	33	39
Sleep problems: severity / impact	90 / 93	81/83	0.77 / 0.80	0.54/0.54	55	49
Left fingers / right fingers	91 / 90	78/73	0.74 / 0.76	0.41/0.32	49 / 53	61/53
Left wrist / right wrist	93 / 97	83/80	0.75 / 0.90	0.43/0.40	35 / 28	39/38
Left elbow / right elbow	96 / 95	87/87	0.79 / 0.78	0.42/0.44	20 / 22	31/27
Left shoulder / right shoulder	94 / 93	82/81	0.81 / 0.77	0.45/0.44	39 / 37	40/40
Left hip / right hip	95 / 95	88/83	0.80 / 0.83	0.52/0.40	28 / 29	29/33
Left knee / right knee	96 / 94	82/82	0.85 / 0.81	0.34/0.46	29 / 33	42/43
Left ankle / right ankle	96 / 95	85/91	0.83 / 0.79	0.47/0.62	28 / 28	27/22
Left toes / right toes	95 / 97	87/88	0.76 / 0.85	0.49/0.56	24 / 22	29/32
Neck / back	90 / 90	81/81	0.73 / 0.74	0.42/0.48	55 / 55	44/48

[1] Retest analysis per item of severity and impact of a symptom (severity: none, mild, moderate, severe; impact: no impact, small impact, moderate impact, large impact) [2] Percentage of patients that had the symptom at least 1 day in the past month at week one and month six [3] For each symptom answering the question: over the past month how much [symptom] have you had? [4] For each symptom answering the question: what impact has this [symptom] had on the ability to carry out daily activities Interpretation of the weighted kappa: 0 = only chance agreement, ≤0.20 = poor, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = good, 0.81-1.00 = excellent / perfect

Responsiveness
Seventy-four individuals had clinical and questionnaire data after a follow-up of 6 months and within 2 weeks apart. Of these, 31 individuals had a VAS pain change of 11 mm and 12 individuals a VAS pain change of 25 mm; equivalent data for VAS fatigue were 32 individuals and 16 individuals (17% missing VAS change scores). The Wilcoxon signed-rank test for the questionnaire items joint pain severity and impact were non-significant when using both cut-off points for VAS pain (p=0.46 and p=0.24 respectively for cut-off 11 mm, and both p=0.43 for cut-off 25 mm). Also, no statistically significant differences were found for fatigue severity/impact for the VAS fatigue (p=0.21 p=0.63 respectively for cut-off 11 mm, and p=0.11 and 0.15 respectively for cut-off 25 mm).

Frequency and impact of symptoms
The frequency of symptoms and their impact in the individuals at risk of RA was high (Table 4). Overall, presence of symptoms was reported more often by the seronegative individuals with CSA, followed by the ACPA-positive and then the RF-positive group (except for fatigue which occurred more often in ACPA-positive individuals). In all three groups the percentage of individuals with symptoms at least 16 days in the past month was highest for joint pain (37-72%), joint stiffness (34-68%), weakness or loss of strength (21-35%) and fatigue (28-39%). The severity and impact were reported similarly across the three groups with the exception of burning and tingling sensations and muscle cramps, which had a lower frequency in ACPA-positive individuals, but a higher impact and severity.

Joint pain was mostly reported in the fingers (ACPA+ 58%, RF+ 52%, seronegative with CSA 65%), however the percentage of neck and back pain was also high (ACPA+ 39%, RF+ 46%, seronegative with CSA 47% and ACPA+ 50%, RF+ 52%, seronegative with CSA 57%, respectively) (Figure 1). Usually, this joint pain was described as aching, symmetric and only one third reported them as mild. The location of joint pain had a similar distribution across all groups.

Finally, we evaluated the pattern of joint pain in the period preceding the first questionnaire (Table 1). Joint pain rapidly increasing and then remaining constant was reported by 9% (pattern A), joint pain gradually increasing over time by 16% (pattern B), and a more intermittent pattern by 53% (respectively 23% and 30% for in between periods without pain (D) and symptoms coming and going but always some pain present (C)) (see Table 4 for classification by antibody positivity or negativity). Fourteen individuals (6%) had missing data. The remainder of the individuals chose the option of either drawing a pattern themselves or describing it. Of those, one was similar to A, five similar to C and 6 to D. Of the remaining 25, six had no symptoms, 11 had a peak in the beginning and then declining symptoms (mostly to zero), three had a combination of the intermittent patterns, one reported an intermittent pattern with no remaining symptoms afterwards, three were unclear and the last individual filled in both A and C.

Table 4: Duration, severity and impact of the SPARRA questionnaire items (N=237, and joint pain patterns (N=223)

Items	Duration				Severity				Impact			
	At least 16 days in the past month				If present, moderate/ severe				If present, moderate/ high impact			
	ACPA+	RF+	Seronegative	FDR	ACPA+	RF+	Seronegative	FDR	ACPA+	RF+	Seronegative	FDR
Joint pain	37	38	72	22	63	74	72	42	51	44	56	42
Joint swelling	7	13	19	6	59	60	54	33	44	67	57	67
Joint stiffness	38	34	68	22	66	65	73	57	48	35	51	29
Burning sensations	11	15	21	6	69	75	60	80	56	63	48	40
Tingling sensations	10	8	19	6	57	78	45	75	43	44	29	25
Numbness	9	4	11	11	53	33	48	100	44	25	39	50
Change in skin colour	3	6	11	17	27	50	50	67	13	17	38	33
Muscle cramps	5	9	7	0	68	46	38	42	37	23	17	0
Weakness or loss of strength	35	21	35	22	64	63	66	50	55	48	52	33
Fatigue	39	28	33	28	84	63	63	64	70	57	53	46
Emotional distress	10	11	19	17	55	42	54	70	49	29	46	30
Concentration difficulties	17	9	14	0	64	56	65	29	56	33	61	14
Sleep problems	27	15	40	22	80	64	72	50	60	36	53	25

*Joint pain patterns**	*Description of the pattern (see also Table 1)*	ACPA+	RF+	Seronegative	FDR
Pattern A	Increased rapidly and then remained constant	6	10	16	11
Pattern B	Gradually increased to their current level over time	15	17	21	6
Pattern C	Come and gone increasing and decreasing though always with some symptoms	30	33	34	22
Pattern D	Come and gone with periods without symptoms in between	32	19	16	28
Pattern E	Own interpretation	17	21	13	33

Data expressed as percentages, * missing data in 14 individuals
Note that the ACPA positive group also includes RF positive individuals, and all RF positive individuals are ACPA negative
Abbreviations: ACPA: anti-citrullinated protein antibodies; RF: rheumatoid factor; CSA: clinically suspect arthralgia; FDR: first degree relatives.

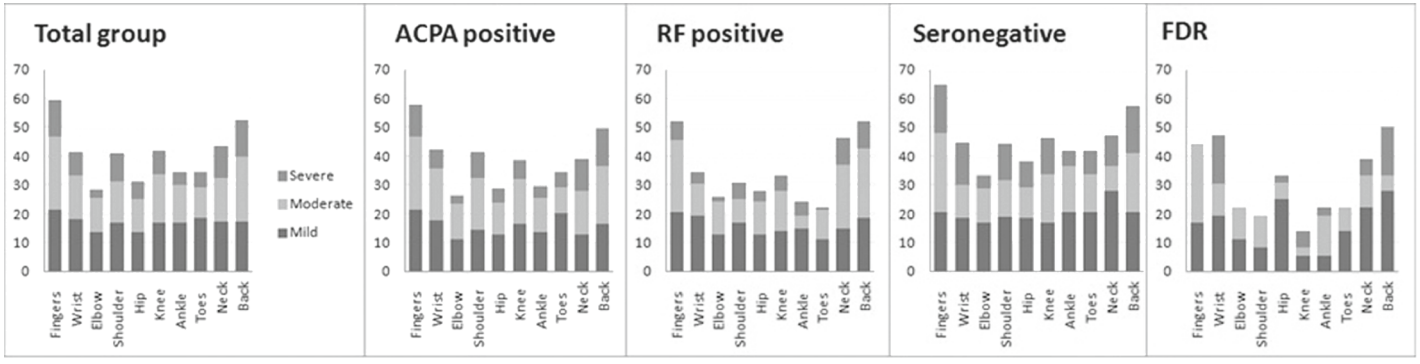


Figure 1: Joint pain location and severity
Reported answers to the following question: "how much pain did you have on average over the last month in mentioned body areas?" Levels of pain ranging from no to severe, expressed as mean percentage of the left and right side of the body. Total group: ACPA and/or RF positive individuals combined with seronegative individuals. First degree relatives (FDR) of patients with RA presented as comparison group.

Comparison with FDR of patients with RA

We compared the questionnaire data with 18 FDR of patients with RA. Missing data in this cohort was very low (1 missing question and 13 questions answered with two options were set on the worst case). The prevalence of symptoms was lower, except for numbness and change in skin colour (Table 4). The location of joint pain was similar for these individuals (Figure 1), just like joint pain patterns A, C and D (11%, 22% and 28%, respectively). The percentage of pattern B was shifted towards the open option where individuals could fill in a pattern themselves (B in 5.6% and other in 33%). Of the latter six individuals, three had no symptoms, two had a peak in the beginning and then no pain, and the last can be set as D.

DISCUSSION

The SPARRA questionnaire has good psychometric properties. The data show a high burden, severity and impact of symptoms in individuals at risk of developing RA.

Evaluation of a complete set of symptoms that might be predictive for RA development is a challenge in the setting of prospective cohort studies, since questions need to be predetermined and are usually based on classification criteria. Using the SPARRA items derived directly from ACPA-positive symptomatic at-risk individuals gave the opportunity to gain more insight into these symptoms.

Questionnaires including symptoms have not yet been reported in cohorts researching individuals at risk of RA. Some use generic tools addressing functional limitations rather than specific symptoms, for example the 36-item Short Form survey (SF-36) and EuroQol five dimensions questionnaire (EQ-5D), which have shown their relevance in patients with RA and other musculoskeletal disease, but not yet in the at risk phase[31-33]. Recently, the use of the Health Assessment Questionnaire (HAQ) has been described in individuals with CSA and it was shown that overall a low score (median 0.5) was present, but a higher score seemed to correlate with inflammation on MRI and arthritis development.[17]

Construct validity and responsiveness of the SPARRA questionnaire were moderate and non-significant (respectively) in this study. The moderate correlation between clinically used VAS scores with questionnaire items can partly be explained by the fact that VAS scores measure symptoms from the past week compared to SPARRA questionnaire items measuring symptoms during the last month. Alternatively, the questions may measure different elements and reflect symptom content not captured with regular VAS pain/global assessment. This would mean that the questionnaire adds information to currently available clinical measures in individuals at risk of RA. The low responsiveness in individuals with changing VAS pain and fatigue scores might relate to the fact that no formal cut-offs are described for this study population and responsiveness could only be measured in a small subgroup. It could be that a change in the questionnaire items is not useful in follow-up of individuals at risk of RA, however future evaluation in a larger population in a longitudinal setting is necessary.

The data showed that besides the expected joint symptoms, the burden of general and nervous system-related symptoms such as burning and tingling sensations, numbness and fatigue was high, especially amongst the ACPA-positive individuals. An explanation could be the presence of a more general subclinical inflammation as was suggested by MRI and PET studies[29 34], as well as an early involvement, prior to subclinical inflammation, of the neurosystem in ACPA-positive individuals as both in-vivo and in-vitro studies have suggested[35 36]. Higher scores in joint pain and joint stiffness in the seronegative individuals might be a consequence of the fact that these individuals were included mainly based on the presence of symptoms. This was underscored by a recent cohort study in which a difference was shown between the symptomatic phase preceding ACPA-positive and ACPA-negative RA, and seronegative individuals had more symptoms at baseline.[15] A lower burden of symptoms in the cohort of FDR of patients with RA was expected as these individuals mostly were without symptoms or antibodies at completion of the baseline SPARRA questionnaire. A similar joint pain location and pattern in the FDR's as compared to the seropositive arthralgia group may reflect their increased risk for RA[16].
For missing data imputation in this study we decided to use a worst case scenario approach. We also checked whether using a best case scenario changed the results, which was not the case (data not shown).

A limitation of the study may be selection bias caused by partly using a convenience sample in which non-response and the associated reasons are lacking. This may have lead to overestimation of the burden of disease, since individuals with more symptoms may be more willing to complete the questionnaire. However, since the (heterogeneous) study population was taken from a set of individuals found in daily practice in secondary care we expect that the results from the study can be generalized to other secondary care practices. Also, 88% of individuals were assessed in a consecutive manner. Another limitation is the fact that comorbidities may influence the reported symptoms and data on comorbidities were not collected for the present study.

The predictive value of the questionnaire items (possibly combined with items of the HAQ) for developing RA requires further investigation. This can hopefully guide item reduction of the questionnaire in the future. Furthermore, responsiveness could also be investigated in medication trials for preventing RA and a longitudinal design would be helpful to assess its use.

In conclusion, this study provides evidence of good psychometric properties of the SPARRA questionnaire, except for moderate construct validity and low responsiveness. In individuals at risk of RA, symptoms are frequent and severe, and have a high impact. Future studies are needed to evaluate whether data from the SPARRA questionnaire can help to improve the prediction of RA.

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SUPPPLEMENTARY MATERIAL

1. Supplementary Figure 1: SPARRA questionnaire english version (only this version added below)
2. Supplementary Figure 2: SPARRA questionnaire dutch version
3. Supplementary Figure 3: SPARRA questionnaire french version
4. Supplementary Figure 4: SPARRA questionnaire german version
5. Supplementary Figure 5: SPARRA questionnaire swedish version
6. Supplementary Table 1: Cohort details
7. Supplementary Table 2: Baseline characteristics all centers
8. Supplementary Table 3: Correlation of the baseline SPARRA questionnaire items with clinical parameters (N=208)

Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) Questionnaire

You may or may not have experienced one or more of the following symptoms in relation to your current episode of joint problems. Please indicate whether you have had the listed symptoms, and when they first appeared. For example, if you have had joint pain for the last 3 months, and swelling for the last 4 years but never had stiffness of your joints, you fill in:

Example of possible symptom	**Experienced (past or present)***	**If yes, how long ago did it first appear?**
Joint pain	Past /present / not	3 months ~~/ years~~
Swelling of the joints	Past /present / not	4 ~~months /~~ years
Stiffness of the joints	Past / present/not	~~... months / years~~

In the bottom two rows, there is space to add any other symptoms you may have noticed.

Possible symptom	**Experienced (past or present)***	**If yes, how long ago did it first appear?***
Joint pain	Past / present / not	… months / years
Swelling of the joints	Past / present / not	… months / years
Stiffness of the joints	Past / present / not	… months / years
Burning sensations in the joints	Past / present / not	… months / years
Tingling sensations in the joints	Past / present / not	… months / years
Numbness in the joints	Past / present / not	… months / years
Changes in skin colour over any joints	Past / present / not	… months / years
Muscle cramps	Past / present / not	… months / years
Weakness or loss of strength	Past / present / not	… months / years
Fatigue	Past / present / not	… months / years
Emotional distress (e.g. sadness, worry, upset)	Past / present / not	… months / years
Concentration difficulties	Past / present / not	… months / years
Sleep problems	Past / present / not	… months / years
Other symptom, namely: _____	Past / present	… months / years
Other symptom, namely: _____	Past/present	… months / years

If you have had any other symptoms **before this episode of joint problems** that you think may be relevant, you can describe them here: _____
_____

The answers to the following questions will help us to understand more about the type of symptoms you have experienced over the **past month.** Do not think too long about the questions; the first answer that comes to mind is often the best.
Please read each question and circle the one option which best answers the question for you, for example:

Example question				
a) Over the past month how many days of the month have you had X in your joints?	0 days (continue to next question)	1 to 5 days	6 to 15 days	16 to 30 days

Q1: Joint pain				
1a) Over the past month how many days of the month have you had pain in your joints?	0 days (continue to question 2)	1 to 5 days	6 to 15 days	16 to 30 days
1b) Over the past month how much joint pain have you had?	None	Mild	Moderate	Severe
1c) What impact has this joint pain had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
1d) Which of the following descriptions is most like your joint pain?	Burning pain	Sharp or stabbing pain	Aching pain	Other type of pain. Please describe: _____ _____
1e) Does your joint pain move from joint to joint?	No	from arms to legs	from legs to arms	from one side to the other

Q2: Joint swelling				
2a) Over the past month how many days of the month have you had swelling in your joints?	0 days (continue to question 3)	1 to 5 days	6 to 15 days	16 to 30 days
2b) Over the past month how much joint swelling have you had?	None	Mild	Moderate	Severe
2c) What impact has joint swelling had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
2d) Where did you feel the joint swelling? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q3: Joint stiffness				
3a) Over the past month how many days of the month have you had stiffness in your joints?	0 days (continue to question 4)	1 to 5 days	6 to 15 days	16 to 30 days
3b) Over the past month, how much joint stiffness have you had?	None	Mild	Moderate	Severe
3c) If you have had joint stiffness when you wake up in the morning how long does it last?	I don't have morning stiffness	For less than an hour: … minutes**	For 1 to 2 hours	All morning
3d) What impact has joint stiffness had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
3e) Where did you feel the joint stiffness? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

** please fill in how many minutes on average

Q4: Burning sensations in joints				
4a) Over the past month how many days of the month have you had a feeling of burning in your joints?	0 days (continue to question 5)	1 to 5 days	6 to 15 days	16 to 30 days
4b) Over the past month, how much feeling of burning in your joints have you had?	Not	Mild	Moderate	Severe
4c) What impact has this feeling of burning in your joints had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
4d) Where did you experience this feeling of burning in your joints? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q5: Tingling sensations in joints				
5a) Over the past month how many days of the month have you had pins and needles or tingling sensations?	0 days (continue to question 6)	1 to 5 days	6 to 15 days	16 to 30 days
5b) Over the past month how much tingling have you had?	None	Mild	Moderate	Severe
5c) What impact has this tingling had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
5d) Where did you feel the tingling? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q6: Numbness				
6a) Over the past month how many days of the month have you had numbness?	0 days (continue to question 7)	1 to 5 days	6 to 15 days	16 to 30 days
6b) Over the past month, how much numbness have you had?	None	Mild	Moderate	Severe
6c) What impact has this numbness had on your ability to carry out activities such as e.g. work, household chores, childcare, social activities?	No impact	A small impact	A moderate impact	severe impact
6d) Where did you feel the numbness? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q7: Changes in skin colour over joints (may be skin looking unusually red, blue, brown, etc)				
7a) Over the past month how many days of the month have you had skin discolouration over any joints?	0 days (continue to question 8)	1 to 5 days	6 to 15 days	16 to 30 days
7b) Over the past month, how much skin discolouration have you had?	None	Mild	Moderate	Severe
7c) What impact has skin discolouration had on your ability to carry out day to day activities?	No impact	A small impact	A moderate impact	A large impact
7d) Where did you experience skin discolouration? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q8: Muscle cramps				
8a) Over the past month how many days of the month have you had muscle cramps?	0 days (continue to question 9)	1 to 5 days	6 to 15 days	16 to 30 days
8b) Over the past month, how much muscle cramping have you had?	None	Mild	Moderate	Severe
8c) What impact have muscle cramps had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
8d) Where did you experience muscle cramps? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q9: Weakness				
9a) Over the past month how many days of the month have you had weakness?	0 days (continue to question 10)	1 to 5 days	6 to 15 days	16 to 30 days
9b) Over the past month, how much weakness have you had?	None	Mild	Moderate	Severe
9c) What impact has weakness had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact
9d) Where did you experience weakness? (circle all that apply)	Hand: One Both	Arm: One Both	Foot: One Both	Leg: One Both

Q10: Fatigue				
10a) Over the past month how many days of the month have you had fatigue?	0 days (continue to question 11)	1 to 5 days	6 to 15 days	16 to 30 days
10b) Over the past month, how much fatigue have you had?	None	Mild	Moderate	Severe
10c) What impact has fatigue had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact

Q11: Emotional Distress (e.g. sadness, worry, upset)				
11a) Over the past month how many days of the month have you had these feelings of emotional distress?	0 days (continue to question 12)	1 to 5 days	6 to 15 days	16 to 30 days
11b) Over the past month, how much emotional distress have you felt?	None	Mild	Moderate	Severe
11c) What impact have these feelings of emotional distress had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact

Q12: Concentration difficulties				
12a) Over the past month how many days of the month have you had difficulties in concentrating?	0 days (continue to question 13)	1 to 5 days	6 to 15 days	16 to 30 days
12b) Over the past month, how much difficulty with concentrating have you had?	None	Mild	Moderate	Severe
12c) What impact have difficulties in concentrating had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact

Q13: Sleep problems				
13a) Over the past month how many days of the month have you had problems sleeping?	0 days (continue to question 14)	1 to 5 days	6 to 15 days	16 to 30 days
13b) Over the past month, how much problem sleeping have you had?	None	Mild	Moderate	Severe
13c) What impact have sleeping difficulties had on your ability to carry out daily activities (e.g. work, household chores, childcare, social activities)?	No impact	A small impact	A moderate impact	A large impact

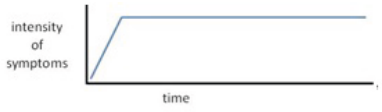
Q 14: Please use the scales below to rate how much pain you had on average over the last month in each of the mentioned body areas. Please circle the number which corresponds with your level of pain with 0 being no pain and 3 being severe pain.

		None	Mild	Moderate	Severe			None	Mild	Moderate	Severe
A	**Left fingers**	0	1	2	3	I	**Right fingers**	0	1	2	3
B	**Left wrist**	0	1	2	3	J	**Right wrist**	0	1	2	3
C	**Left elbow**	0	1	2	3	K	**Right elbow**	0	1	2	3
D	**Left shoulder**	0	1	2	3	L	**Right shoulder**	0	1	2	3
E	**Left hip**	0	1	2	3	M	**Right hip**	0	1	2	3
F	**Left knee**	0	1	2	3	N	**Right knee**	0	1	2	3
G	**Left ankle**	0	1	2	3	O	**Right ankle**	0	1	2	3
H	**Left toes**	0	1	2	3	P	**Right toes**	0	1	2	3
Q	**Neck**	0	1	2	3	R	**Back**	0	1	2	3

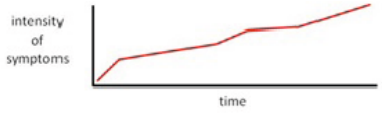
Q15: This question asks about how your symptoms have developed **since the time they first began.** Please select the ***one pattern of the following options that you think best describes*** how your symptoms have developed. Between the time that your symptoms first began and now, have your symptoms:

Please tick the *one* box which best describes your symptom pattern:

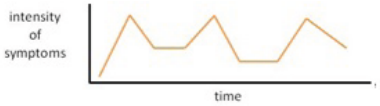
a) increased ***rapidly*** **and then remained constant** (like the line to the right):



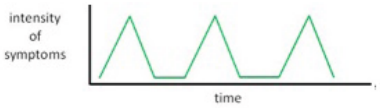
b) ***gradually*** increased to their current level over time (like the line to the right):



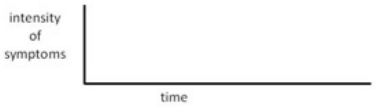
c) **come and gone** increasing and decreasing though ***always with some symptoms*** (like the line to the right):



d) **come and gone** with ***periods without symptoms*** **in between** (like the line to the right):



e) If these do not match your symptom experience, use the space to the right to draw what your symptoms were like between the time they began and now, or describe them below:



_____

_____

_____

Is there anything else you want to tell us about your symptoms?
Or do you want to tell us anything about the questionnaire?
Please leave your comments below:

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Thank you very much!

Supplementary Table 1: Cohort details

Center (number of inclusions) Study name (if applicable)	Reference	In- and exclusion criteria, and other relevant cohort information
Netherlands (N=125) Prevention study	Bos et al, 2010[20]	**Inclusion:** patients with muskuloskeletal symptoms testing positive for ACPA and/or RF referred by primary care physicians, or presence of clinically suspect arthralgia defined by a rheumatologist. **Exclusion:** arthritis revealed by chart review or baseline physical examination; previous treatment with a disease-modifying antirheumatic drug (except hydroxychloroquine which was allowed); recent glucocorticoid treatment (past 3 months); participants of a randomised placebo-controlled trial with the goal of preventing arthritis development with dexamethasone; erosions on hand or feet x-ray examination. **Patient selection:** consecutive patients from the cohort, clinical databank was checked regularly to inform auto-antibody positive individuals that were not yet included in the cohort and ask for their participation. **Medication use:** 3 individuals used hydroxychloroquine prior to inclusion in the cohort, no other disease-modifying antirheumatic drugs were used, and non used corticosteroids.
United Kingdom (N=69)	NA[2]	**Inclusion:** patients with clinically suspect arthralgia defined by a rheumatologist. **Exclusion:** arthritis revealed by baseline physical examination; previous treatment with a disease-modifying antirheumatic drug (including hydroxychloroquine) **Patient selection:** consecutive patients from the cohort. **Medication use:** no disease-modifying antirheumatic drugs or glucocorticoids were used.
Sweden (N=15) Risk-RA Karolinska cohort	NA[2]	**Inclusion:** patients with muskuloskeletal symptoms testing positive for ACPA referred by primary care physicians (or other specialist) to the rheumatologist . **Exclusion:** presence of arthritis on either clinical examination or ultrasound examination by rheumatologist. Previous history or diagnosis of arthritis or other rheumatological diseases. **Other:** in the cohort usually arthritis is diagnosed as either clinical synovitis and/or ultrasound examination. For current study we have taken only the clinical diagnosis as valid. **Patient selection:** consecutive patients from the cohort. **Medication use:** none of the individuals used disease-modifying antirheumatic drugs or corticosteroids.

table continues

Center (number of inclusions) Study name (if applicable)	Reference	In- and exclusion criteria, and other relevant cohort information
Austria (N=10)	NA[2]	**Inclusion:** seropositive and seronegative arthralgia patients (rheumatologist confirmed) from the outpatient clinic of the Department of Internal Medicine III, Division of Rheumatology, at the Medical University of Vienna **Exclusion:** persons with arthritis; persons who do not sufficiently speak the local language; who cannot fill out a questionnaire or who do not give informed consent. **Patient selection:** patients were detected from a "pre-arthritis" cohort and through the clinical databank. **Medication use:** detailed data was not collected.
Switzerland (N=18)[1] SCREEN-RA cohort	Finckh et al, 2011[21]	**Inclusion:** being a first degree relative of an RA patient (rheumatologist confirmed and treated). **Exclusion:** arthritis at baseline (study nurse examination for screening, in case of doubt a rheumatologist is asked to come and check); other established inflammatory rheumatic disease (i.e. concomitant SLE would be an exclusion). **Other:** in the cohort usually arthritis is diagnosed as either clinical synovitis and/or ultrasound examination. For current study we have taken only the clinical diagnosis as valid. **Patient selection:** the SCREEN-RA cohort includes FDR of patients with RA from multiple centers in Switzerland. Consecutive patients from the cohort were included, for the present study only the Geneva site was used. **Medication use:** detailed data was not collected.

[1] Switzerland was used as comparison cohort
[2] Data from the UK, swedish and austrian cohorts have not been published yet

Abbreviations: NA: not applicable, ACPA: anti-citrullinated protein antibodies, RF: rheumatoid factor; SLE: systemic lupus erythematosus; FDR: first-degree relatives; RA: rheumatoid arthritis, UK: United Kingdom

Supplementary Table 2: Baseline characteristics all centers

Variable	Netherlands (N=125)	United Kingdom (N=69)	Sweden (N=15)	Austria (N=10)	Switzerland (N=18)[1]
Age[2]	50 (12.4)	46 (14.1)	51 (15.1)	52 (12.6)	57 (9.5)
Males (%)	33	30	7	20	11
Symptom duration (months)[3]	30 (12-60)	11 (5-38)	20 (7-46)	ND	22 (7-51)
Tender Joint Count (44 joints)	1 (0-2)	7 (3-17)	0 (0-0)	0 (0-1)	1 (0-3)
VAS pain (mm)[3]	23 (2-55)	49 (22-74)	36 (6-66)	11 (1-32)	ND
VAS patient global assessment (mm)[3]	25 (1-51)	47 (12-66)	32 (10-60)	18 (1-50)	ND
VAS fatigue	48 (8-69)	66 (33-86)	55 (1-75)	11 (2-33)	ND
Current smoking (%)	25	19	13	22	17
FDR with RA (%)[3]	22	40	14	13	100

table continues

Variable	Netherlands (N=125)	United Kingdom (N=69)	Sweden (N=15)	Austria (N=10)	Switzerland (N=18) [1]
Autoantibody status					
Only ACPA positive (%)	18	10	47	20	33
Only RF positive (%)[3]	32	12	0	50	6
ACPA and RF positive (%)	39	20	53	0	0
Seronegative (%)	11	58	0	30	61

[1] Switzerland was used as comparison cohort
[2] Mean (standard deviation; SD), all other continuous variables mentioned as median (25^{th}-75^{th} percentile)
[3] Missing values; 11% for VAS fatigue, 10% for VAS pain and global assessment, 7% for symptom duration, 2% for family history, and
one patient for RF (marked as ACPA positive only now)

Abbreviations: VAS: visual analogue scale; FDR: first degree relative; RA: rheumatoid arthritis; ACPA: anti-citrullinated protein antibodies; RF: rheumatoid factor; ND: not done.

Supplementary Table 3: Correlation of the baseline SPARRA questionnaire items with clinical parameters (N=208)

Questionnaire item	VAS pain	VAS global	VAS fatigue
Joint pain severity[1]	0.63[3]		
Joint pain impact[2]	0.58[3]	0.61	
Joint swelling impact		0.38	
Joint stiffness impact		0.58	
Fatigue severity			0.61[3]
Fatigue impact			0.58[3]
Emotional distress impact		0.44	
Sleep problems impact		0.49	

Correlations expressed as Spearman's rank correlation coefficients, all p-values were <0.01
Visual Analogue Scale (VAS) scores used as continuous data

[1] Severity categories: none, mild, moderate, severe (applicable to all questionnaire items)
[2] Impact categories: no, small, moderate, large impact (applicable to all questionnaire items)
[3] Measuring concurrent validity (versus those without asterisk measuring construct validity)

CHAPTER

6

Frequency and timing of musculoskeletal symptoms, infections and comorbidities in future inflammatory arthritis patients, a Dutch primary care database study

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ABSTRACT

Objectives: Little is known about relevant events in the at-risk phase of rheumatoid arthritis before the development of clinically apparent inflammatory arthritis (IA). The present study was undertaken to identify the frequency and timing of musculoskeletal symptoms, infections and comorbidity in future IA patients.

Methods: In a nested case-control study using electronic health records of general practitioners, the frequency and timing of 192 symptoms or diseases were evaluated before a diagnosis of IA, using the the International Classification of Primary Care coding system. Cases were 2406 adults with a new diagnosis IA between 2012 and 2016; controls were matched 1:2. The frequency of primary care visits was compared using logistic regression in different time periods before IA diagnosis.

Results: The frequency of primary care visits for musculoskeletal symptoms (mostly of shoulders, wrists, fingers and knees) was significantly higher in IA patients versus controls within the final 1.5 years before diagnosis, with odds ratios of 3.2 (CI 2.8-3.5), 2.8 (CI 2.5-3.1) and 2.5 (CI 2.2-2.8) at 6, 12 and 18 months before diagnosis, respectively. Also, infections, IA-comorbidities and chronic diseases were more prevalent in cases than controls, but more evenly spread out over the whole 6-year period before IA.

Conclusions: There was an increased frequency of primary care visits for musculoskeletal symptoms, infectious diseases and comorbidities prior to the diagnosis of IA. This diverging trend is present for 4-6 years, but becomes significant around 1.5 years before the diagnosis. Validation of these results is warranted.

Rheumatoid arthritis (RA) is usually diagnosed shortly after the appearance of clinically apparent inflammatory arthritis (IA). The time between the onset of persistent joint symptoms and the diagnosis RA by the rheumatologist varies[1]; in the Netherlands the median duration is four months[2 3]. Early recognition improves the outcome[3]. General practitioners (GPs) can play an essential role in earlier detection of IA as they are the first professional to be consulted for health problems and all Dutch inhabitants are listed with a GP. Furthermore, the GP has a gatekeeper role and therefore refers a patient with suspected IA to the rheumatologist. They have a complete overview of all health problems in their electronic health records (EHRs). The unique health care system in the Netherlands makes it possible to study symptom and morbidity patterns before the diagnosis.

It appears that GPs mostly use classical signs of inflammation such as pain and swelling to identify those with a high probability of having IA, and that those signs are the triggers for referral to secondary care[4]. However, additional symptoms or conditions may occur before the diagnosis that are at that time not attributed to emerging RA, but do lead to increased ambulatory care utilization[5]. This is underscored by a higher rate of sick leave already eight months before the first prescription of antirheumatic drugs[6]. Also, the number of comorbid diseases at the onset of IA is higher than in a control group, however, it is not clear whether these diseases were already present before the onset of IA[7].

In the phase before clinical RA, subclinical autoimmunity and inflammation often occur for several years[8 9]. This may be related to the influence of environmental factors, such as infections or life style factors[10]. However, little is known about symptoms, pathogenetic events, other diseases and their timing during this phase[11]. Also, available clues mostly come from case-control studies and studies of at-risk populations. These studies have the limitation that only selected groups of individuals such as those positive for anti-citrullinated protein antibodies (ACPA) or first-degree relatives of patients with RA are studied, usually after referral to secondary care because of more severe symptoms[11 12]. Therefore, there is a need to also study the at-risk phase of RA in the unselected primary care setting.

The present study focuses on preexisting symptoms and diseases that are possibly related to RA with the goals to improve early identification of future IA patients and to identify possible pathogenetic clues. Data from EHRs of GPs from a large Dutch national database was used to answer the following research questions: 1) To what extent are musculoskeletal symptoms, infections and/or RA-related comorbidities more prevalent before the diagnosis IA compared to control patients? 2) What is the lead time between these early symptoms or disorders and the diagnosis IA?

PATIENTS AND METHODS

Study population

Data was used from Nivel Primary Care Database (Nivel-PCD)[13]. Nivel-PCD collects data from routine EHR systems from a representative sample of approximately 500 general practices with a total of more than 1.5 million registered patients, including information about consultations, morbidity, prescriptions and diagnostic tests. Diagnoses were recorded using the International Classification of Primary Care (ICPC-1) coding system[14]. Only data with sufficient quality was used: GPs had to have recorded data at least 46 weeks of the year with at least 70% ICPC coded visits. Adult patients (≥ 18 years) were selected based on having a new diagnostic code of IA (ICPC code L88) in the years 2012 to 2016, hereby identifying only incident cases with at least 1 year (with a maximum of 6 years) retrospective follow-up. L88 includes RA, psoriatic arthritis and ankylosing spondylitis[15]. In case the start date of IA was preceded by the prescription of a disease-modifying antirheumatic drug (DMARD) and/or biological, we assumed that documentation of the L88 code might have been delayed and the date of diagnosis was set on the start date of the first DMARD or biological. Selection included: methotrexate, leflunomide, sulfasalazine, abatacept, rituximab, etanercept, infliximab, adalimumab, certolizumab, golimumab, tocilizumab, anakinra, ustekinumab. Use of hydroxychloroquine was allowed before diagnosis of IA since this is prescribed occasionally in the at-risk phase in patients not having arthritis. Each case was matched with 2 controls (without IA in the past) in the same general practice based on age (+/- 3 years), gender and duration of follow-up (depending on registration date of the patient in a general practice, and registration of that particular general practice in Nivel-PCD).

Procedures

We used data from EHRs containing information on consultations and prescriptions before the IA-date or matched end date of the control patients in the period 2006 to 2016. Consultations are mostly physical visits of patients to the GP, but can also be consultations by telephone or a debrief from a secondary care specialist. Throughout the rest of the manuscript the term primary care visits is used. Prescriptions are those started by the GP as well as repeat prescriptions of medication started in secondary care. We preselected a list of 192 ICPC codes (**Supplementary Table 1**) deemed relevant to RA development, which included musculoskeletal symptoms, infectious diseases and RA-related comorbidities. This selection was based on biological plausibility, literature research[11 16] and expert opinion. In Nivel-PCD comorbidities and chronic diseases are coded seperately, as comordities can be diagnosed more than once and chronic diseases only once.

The study was approved according to the governance code of Nivel-PCD, under number NZR-00314.045. Dutch law allows the use of EHRs for research purposes under certain conditions. According to this legislation, obtaining informed consent nor approval by a medical ethics committee are obligatory for this type of observational studies containing no directly identifiable data (Dutch Civil Law, Article 7:458).

Statistical analysis

We first describe the presence of ICPC codes from the four predermined groups (musculoskeletal symptoms, infections, RA-related comorbidities and chronic diseases) in the individuals with and without a diagnosis of IA. We therefore marked per quartile of the year whether a person was given an ICPC code from a particular group or not, and then summed all the cases which were coded (one or more times) into percentages of the total number of individuals that had retrospective follow-up in that quartile. Per group, based on these numbers we calculated odds ratios (ORs; with 95% confidence intervals, CI, and p-values) of developing IA using univariable logistic regression analysis within the time periods 6, 12 and 18 months prior to the diagnosis (or the matched end date in case of the control individuals).

Next, we performed two different approaches to predict the development of IA based on the ICPC codes within the 12 month period preceding IA. 1) *Using univariable and multivariable logistic regression analyses.* The univariable analysis was corrected for multiple testing using false positive rate control[17]. Codes from the group of infections were combined into 11 groups (see **Supplementary Table 1**), since the frequency of individual ICPC codes was too low for analysis. A backwards stepwise approach was used for the multivariable analysis, ultimately leaving only those ICPC codes with a p-value <0.05. This led to one multivariable prediction model containing the ICPC codes from all groups and the diagnostic performance was described using the area under the curve (AUC) of the receiver operating curve. Age and gender were included irrespective of their significance level.

2) *Using Classification and Regression Tree (CART) analysis*[18]. This nonparametric statistical procedure uses hierarchical variable selection to create a decision tree, and thereby creates the best and most simple combination of variables to predict a certain outcome. In short, it examines all splitting variables (ICPC codes) and first selects the best predictor for the outcome (IA diagnosis). This process is repeated and the next steps will include the prior steps, i.e. step 2 is the best predictor given the fact that the answer in the first step was taken into account, and so on. We used this approach, because it resembles the way that a general practitioner evaluates a certain patient.

Univariable and multivariable regression analyses were performed with Stata/MP 13.0 (StataCorp, College Station, TX, USA). For CART analysis we used SPSS version 21 (IBM Corp, Armonk, NY, USA).

RESULTS

Patient characteristics

In total, 2314 IA cases with a retrospective follow-up of at least one year could be matched to 4541 controls (see flowchart in **Figure 1**) from 262 practices. For 23 cases no controls could be matched. The mean age for cases was 57.6 years (interquartile range, IQR, 24), compared to 56.6 years (IQR 23) in the control group. Both groups contained more women than men (60%).

FIGURES

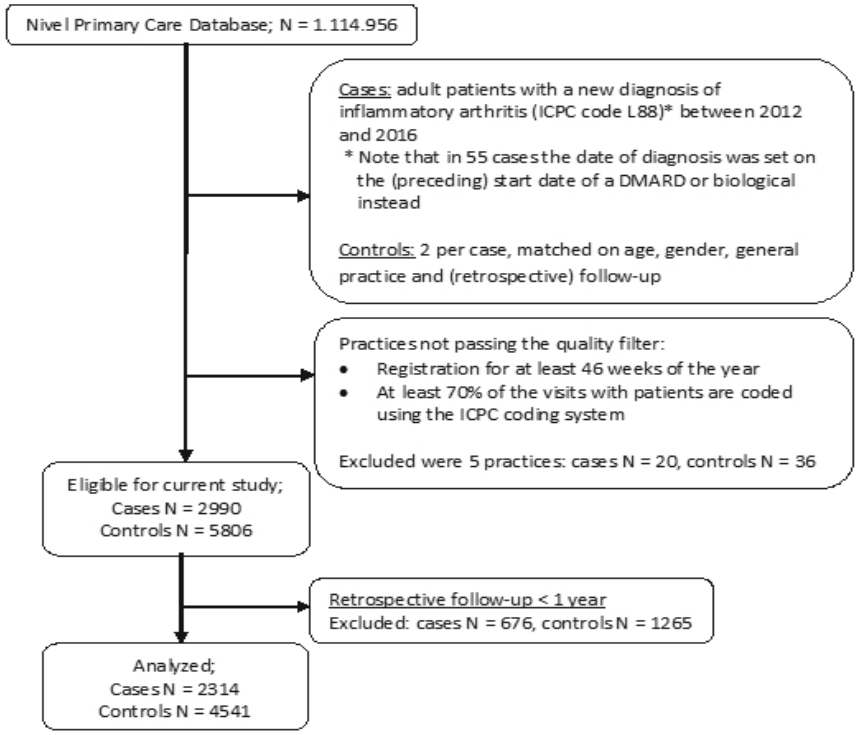


Figure 1. Flowchart of inclusion

Frequency of primary care visits prior to IA diagnosis

In patients receiving the diagnosis of IA, the GP more frequently coded symptoms or diseases related to the musculoskeletal system than in control patients (**Figure 2**). A diverging trend is already visible 4-6 years before the diagnosis, but becomes more pronounced in the final 1.5 years. ORs for the development of IA were 3.2 (CI 2.8-3.5, $p<0.05$), 2.8 (CI 2.5-3.1, $p<0.01$) and 2.5 (CI 2.2-2.8, $p<0.01$) at 6, 12 and 18 months prior to the diagnosis, respectively. Noteworthy is that the number of persons with retrospective follow-up

decreased the further from the IA diagnosis or end point in the controls. For cases/controls these numbers were 2314/4541, 1749/3439, 730/1430, 172/341, respectively, at 1, 2, 4 and 6 years. However, the differences between cases and controls remain present over the entire period.

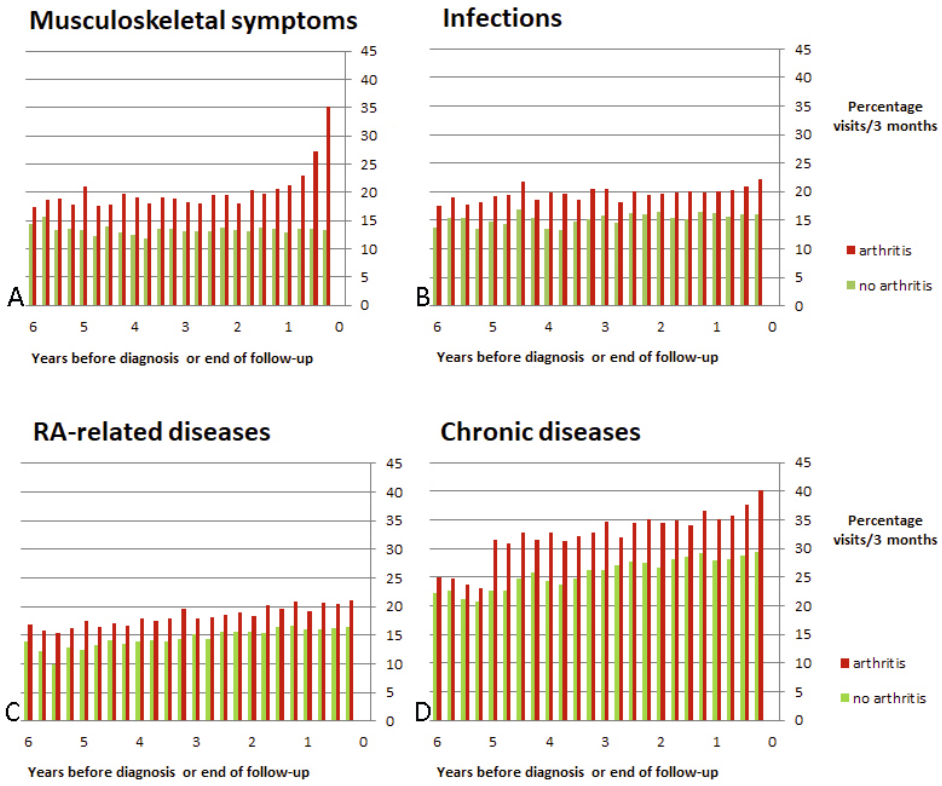


Figure 2. Recorded ICPC codes by the general practitioner (GP) within four groups of symptoms/diseases A) musculoskeletal symptoms, B) infections, C) inflammatory arthritis related diseases and D) chronic diseases. One or more visits per 3 months within a patient was counted as 1 visit, this was then divided by all patients having follow-up at that time point

Data on infections, RA-related comorbidities and chronic diseases showed a less clear pattern over time, although the higher frequency in cases than in controls seems to be present over the entire time period of six years. The ORs for infections were 1.4 (CI 1.3-1.6, p<0.01), 1.5 (CI 1.3-1.6, p<0.01) and 1.5 (CI 1.3-1.7, p<0.01) at 6, 12 and 18 months, respectively. For RA-related comorbidities these numbers were 1.3 (CI 1.2-1.5, p<0.01) for all time points, and for chronic diseases 1.7 (CI 1.5-1.8, p<0.01), 1.7 (CI 1.5-1.9, p<0.01) and 1.7 (CI 1.6-1.9, p<0.01) at 6, 12 and 18 months, respectively.

Individual ICPC-1 codes and their relation with IA development

Univariable logistic regression analyses showed an abundance of ICPC codes across all four groups that were statistically significantly related to the development of IA. As expected from the results shown in **Figure 2**, most of these ICPC codes came from the musculoskeletal system. **Table 1** shows the most predominant relations (ORs ≥ 2.4) (for a complete overview see **Supplementary Table 2**). The most frequent symptomatic joints were the shoulders, wrists, fingers and knees. Also, carpal tunnel syndrome was more frequently present in IA cases. Notably, specific infections were not found to be increased in future IA patients. The main associated recorded chronic diseases in future IA patients were psoriasis, inflammatory bowel disease and gout (the former two as expected due to the definition of IA).

Table 1. Univariable logistic regression analysis of the relation of individual ICPC codes with IA development

ICPC	Description	Group	OR	CI	p-value
L20	Joint symptom/complaint NOS	Musculoskeletal	8.1	5.8 - 11.3	<0.01
L97	Chronic internal derangement knee	Musculoskeletal	5.9	1.6 - 21.8	<0.01
L11	Wrist symptom/complaint	Musculoskeletal	4.9	3.2 - 7.5	<0.01
NA	Other infectious symtoms	Infections	4.9	1.5 - 15.7	<0.01
L12	Hand/finger symptom/complaint	Musculoskeletal	4.0	3.1 - 5.1	<0.01
S91	Psoriasis	Chronic disease	3.7	2.5 - 5.4	<0.01
D94	Chronic enteritis/ulcerative colitis	Chronic disease	3.5	1.9 - 6.4	<0.01
T92	Gout	Chronic disease	3.5	2.6 - 4.7	<0.01
L29	Symptom/complaint musculoskeletal other	Musculoskeletal	2.9	1.9 - 4.4	<0.01
N93	Carpal tunnel syndrome	Musculoskeletal	2.7	1.9 - 4.0	<0.01
L92	Shoulder syndrome	Musculoskeletal	2.6	2.0 - 3.4	<0.01
L91	Osteoarthrosis other	Chronic disease	2.6	1.9 - 3.5	<0.01
L19	Muscle symptom/complaint NOS	Musculoskeletal	2.5	1.6 - 4.0	<0.01
B80	Iron deficiency anaemia	RA-related diseases	2.4	1.6 - 3.6	<0.01
B81	Anaemia, Vitamin B12/folate deficiency	RA-related diseases	2.4	1.5 - 3.6	<0.01

After correction for multiple testing using false positive rate control none of these variables lost their significance

Abbreviations: OR: odds ratio; CI: 95% confidence interval; NA: not applicable; NOS: not otherwise specified; IA: inflammatory arthritis

We then used all ICPC codes to build a multivariable prediction model for IA development using data within 12 months prior to this diagnosis. The AUC of this model was 0.69. **Table 2** shows the top 10 ICPC codes (for the complete prediction model containing 32 ICPC codes and age/gender see **Supplementary Table 3**). The top ten includes both joint symptoms (general, wrist, hand and schoulder) as well as more specific diagnoses such as psoriasis accompanying psoratic arthritis and chronic enteritis/ulcerative colitis accompanying ankylosing spondylitis.

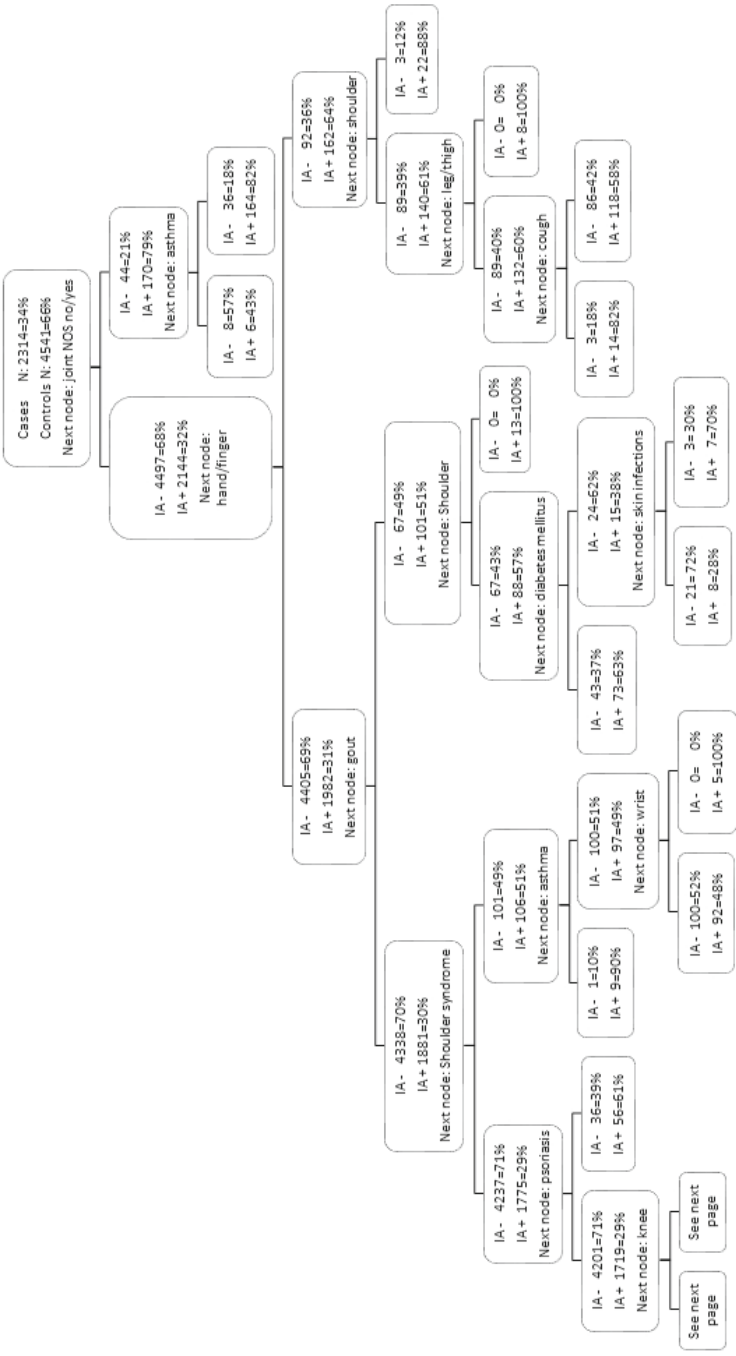
Table 2. Multivariable logistic regression analysis of the relation of individual ICPC codes with IA development (N=2314 cases and N=4541 controls)

ICPC	Description	Group	OR	CI	p-value	Obs*
L20	Joint symptom/complaint NOS	Musculoskeletal	7.9	5.5 - 11.1	<0.01	170/44
L97	Chronic internal derangement knee	Musculoskeletal	5.0	1.3 - 19.5	0.02	9/3
L11	Wrist symptom/complaint	Musculoskeletal	3.8	2.4 - 6.1	<0.01	73/30
S91	Psoriasis	Chronic diseases	3.8	2.5 - 5.8	<0.01	71/39
L12	Hand/finger symptom/ complaint	Musculoskeletal	3.3	2.5 - 4.4	<0.01	179/94
D94	Chronic enteritis/ulcerative colitis	Chronic diseases	3.0	1.6 - 5.6	<0.01	30/17
T92	Gout	Chronic diseases	2.8	2.0 - 3.9	<0.01	119/69
L92	Shoulder syndrome	Musculoskeletal	2.2	1.6 - 2.9	<0.01	137/106
B80	Iron deficiency anaemia	RA-related diseases	2.1	1.4 - 2.7	<0.01	56/46
N93	Carpal tunnel syndrome	Musculoskeletal	2.0	1.3 - 3.0	<0.01	66/48

* Observations of number of patients (left: cases/right: controls) with that ICPC code within the last 12 months Abbreviations: OR: odds ratio; CI: 95% confidence interval; NA: not applicable; NOS: not otherwise specified; IA: inflammatory arthritis

Classification and regression trees (CART)

A CART analysis was performed to find the best and most simple combination of ICPC codes to predict IA. The tree is shown in **Figure 3**. The AUC was 0.64. The classification tree starts with an a priori probability of 34% of developing IA in this dataset (predefined based on the matching process). Thereafter, all nodes residing to the right indicate the symptom mentioned in the node above is present and all nodes going to the left indicate the symptom is not present. For example, the chance of developing IA would be raised to 82% if a person has both “joint pain not otherwise specified” and “asthma”. On the other hand, the absence of a certain variable can also lower the chance of developing IA.


Cases N: 2314=34%
Controls N: 4541=66%
Next node: joint NOS no/yes
IA - 4497=68%
IA + 2144=32%
Next node: hand/finger
IA - 44=21%
IA + 170=79%
Next node: asthma
IA - 8=57%
IA + 6=43%
IA - 36=18%
IA + 164=82%
IA - 92=36%
IA + 162=64%
Next node: shoulder
IA - 89=39%
IA + 140=61%
Next node: leg/thigh
IA - 3=12%
IA + 22=88%
IA - 89=40%
IA + 132=60%
Next node: cough
IA - 0= 0%
IA + 8=100%
IA - 3=18%
IA + 14=82%
IA - 86=42%
IA + 118=58%
IA - 4405=69%
IA + 1982=31%
Next node: gout
IA - 67=49%
IA + 101=51%
Next node: Shoulder
IA - 67=43%
IA + 88=57%
Next node: diabetes mellitus
IA - 0= 0%
IA + 13=100%
IA - 43=37%
IA + 73=63%
IA - 24=62%
IA + 15=38%
Next node: skin infections
IA - 21=72%
IA + 8=28%
IA - 3=30%
IA + 7=70%
IA - 4338=70%
IA + 1881=30%
Next node: Shoulder syndrome
IA - 4237=71%
IA + 1775=29%
Next node: psoriasis
IA - 101=49%
IA + 106=51%
Next node: asthma
IA - 36=39%
IA + 56=61%
IA - 1=10%
IA + 9=90%
IA - 100=51%
IA + 97=49%
Next node: wrist
IA - 4201=71%
IA + 1719=29%
Next node: knee
See next page
See next page
IA - 100=52%
IA + 92=48%
IA - 0= 0%
IA + 5=100%

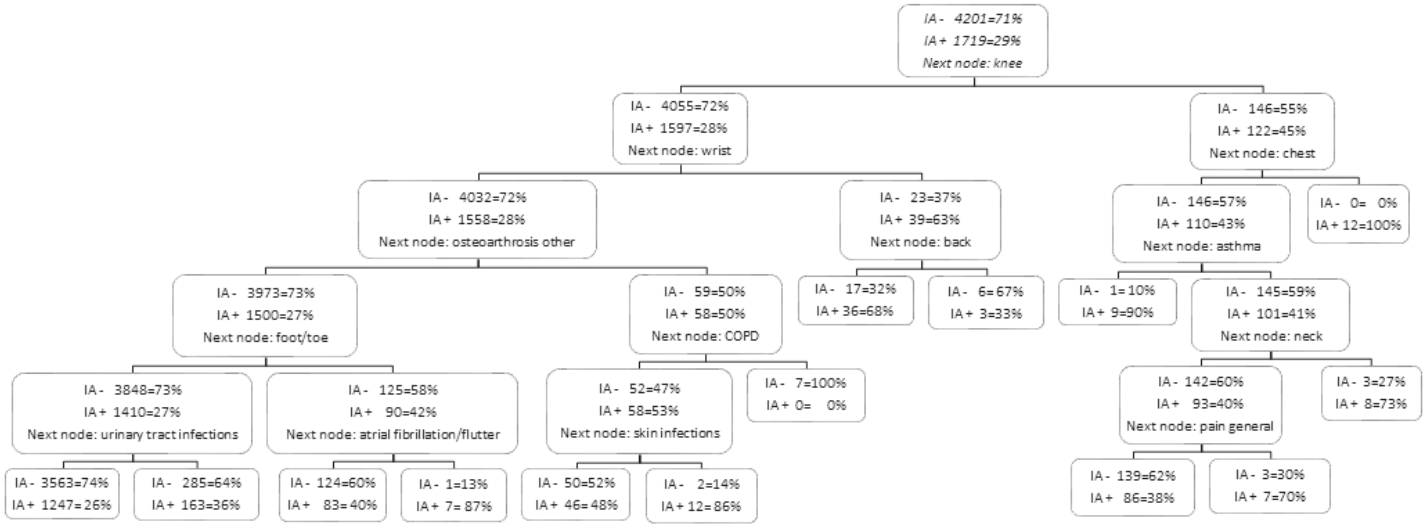


Figure 3. Classification and regression tree (CART) analysis. A CART analysis was performed to find the best and most simple combination of ICPC codes to predict inflammatory arthritis (IA). The a priori probability of developing IA is 34% in this dataset, thereafter all nodes residing to the left indicate the symptom in the node above is not present and all nodes going to the right indicate the symptom is present (with coinciding percentages of developing IA)

DISCUSSION

This study showes an increased frequency of musculoskeletal symptoms preceding the assumed IA-diagnosis date, mainly in the final 1.5 years. Infections, RA-related comorbidities and chronic diseases also were more prevalent in cases than in controls, however, that trend was less clear and more evenly spread out over the whole study period of 6 years. All recorded symptoms and diseases were assembled in a classification tree resembling the way a GP would detect patients to refer to the secondary health care system, but with its low AUC of 64% it needs to be validated before use in clinical practice.

The present results are in line with those of another study in which ambulatory medical care utilization was highest in the two years preceding RA[5]. As in our study, this was mainly attributed to diseases of the musculoskeletal system and connective tissue, although not further specified. We found high associations of the following symptoms/locations: knee, wrist, hand/finger, shoulder and carpal tunnel syndrome. The fact that IA usually starts with symptoms in hands, feet or shoulders was already known[19], but the present data suggest that GPs should also consider emerging IA if patients with chronic problems of the knee or carpal tunnel syndrome visit their practice.

One of the early events in RA pathogenesis appears to be inflammation or infection of mucous membranes, such as in the gums, lung or gut[20-26]. Rather than a one-time initiating event, the present data supports a longer-term exposure, as infections as a total group were more prevalent in cases than controls during the complete follow-up. This also contradicts the finding that recent infections would have a protective effect[27], but complements data that simultaneous development of auto-immunity and an acute phase reaction appear 4-5 years before the diagnosis of RA[8 28 29]. Infections were combined into 11 groups, of which only genital infections, urinary tract infections, and general viral/bacterial infections were significantly related to IA in multivariable analysis (to our knowledge not linked to RA before), with low ORs of 1.4-1.5.

Comorbidities of IA have been studied extensively[30-34]. Seventy percent of patients was found to have at least one chronic disease at onset of IA, which was 10% more than in control patients[7]. We also found more RA-related comorbities and chronic diseases in cases (ORs of 1.3-1.7). Main contributors were psoriasis, chronic enteritis/ulcerative colitis, gout, iron deficiency anemia, vitamin B12/folate deficiency anemia, asthma and diabetes mellitus. Gout hypothetically showed a higher association due to ICPC misclassification, as gout and IA have many similarities[35]. To our knowledge the other contributing factors have not been described before in the pre-disease phases, but only in the phase of established RA, psoriatic arthritis and ankylosing spondylitis[31 34 36-38]. On the other hand, we did not find an (expected) association with osteoarthritis[39] and cardiovascular disease[40] and it thus remains unclear when the excess risk of osteoarthritis and cardiovascular disease starts[41].

Several musculoskeletal symptoms, infections and comorbidities that were more frequently found in IA cases as compared to control patients, have not been previously described in

the phase before IA. This information can help GPs to earlier select individuals at higher risk for developing IA and thus aid in earlier referral. However, the results are not robust enough to support the implementation of a prediction rule for IA in the EHRs of the GPs, without further validation studies.

Our study has some limitations. First, validity of the results for the outcome IA may be lower than compared to studies in which the diagnosis of RA is supported by fulfilment of classification criteria. By definition, the present results are partly generated by patients with psoriatic arthritis or ankylosing spondylitis, the other two constituents of IA. However, the mean age of 57 years and preponderance of females strongly suggest that the IA group mainly consisted of RA patients. Further, the diagnosis of IA is difficult for GPs to make, since it has a relatively low frequency (estimated 6 out of 400 patients with joint symptoms)[42]. This is exemplified by the fact that the IA diagnosis in a prior study has been proven to be about 71% accurate after chart review[15]. However, this is not entirely a bad thing, since it merely reflects the GP's way of evaluating patients. It is their job to differentiate patients that need referral to secondary care from those that do not, and all IA patients benefit from early detection. Secondly, a time lag could exist between the diagnosis IA by the GP and by the rheumatologist. In this large cohort it was not feasible to perform a full chart review including free text fields in the EHRs to correct this. Thirdly, because of the limitations of our data source, no radiographic reports, autoantibody data, or personal habits such as smoking were available. Finally, the a priori chance of developing IA in this case-control study was 34%, in contrast to a prevalence of 0.5-1% for RA in the general population[43]. Therefore it is warranted to perform external validation of the study results in an unselected primary care setting. Future further classification of IA may help to unravel more details on the specific diseases that form subclassifications of the L88 ICPC code. Also, future development of the coding systems in EHRs, including for instance certain algorithms, may make diagnoses more certain and prevent a delay in recording[15 44 45].

In conclusion, musculoskeletal symptoms, infections and comorbidities were more frequent in future IA patients than controls in the years preceding diagnosis. Primary care data, mainly on specific ICPC codes recording 'new' musculoskeletal symptoms such as shoulder pain, chronic pain in the knee and carpal tunnel syndrome, may help GPs to earlier detect and refer patients who will develop IA within 1.5 years. Also, a higher frequency of iron deficiency anemia, vitamin B12/folate deficiency anemia, asthma, diabetes mellitus, genital infections, urinary tract infections and general viral/bacterial infections have not been described before to preceed the development of IA. Future validation of the ICPC codes most associated with IA development is warranted.

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Supplementary Table 1. ICPC-1 codes in four different groups

Musculoskeletal symptoms/disorders					
A01	Pain general/multiple sites	L10	Elbow symptom/complaint	L19	Muscle symptom/complaint NOS
A80	Trauma/injury NOS	L11	Wrist symptom/complaint	L20	Joint symptom/complaint NOS
L01	Neck syptom/complaint	L12	Hand/finger symptom/complaint	L29	Symptom/complaint musculoskeletal other
L02	Back symptom/complaint	L13	Hip symptom/complaint	L87	Ganglion joint/tendon
L03	Low back symptom/complaint	L14	Leg/thigh symptom/complaint	L92	Shoulder syndrome
L04	Chest symptom/complaint	L15	Knee symptom/complaint	L93	Tennis elbow
L07	Jaw symptom/complaint	L16	Ankle symptom/complaint	L97	Chronic internal derangement knee
L08	Shouder symptom/complaint	L17	Foot/toe symptom/complaint	N93	Carpal tunnel syndrome
L09	Arm symptom/complaint	L18	Muscle pain	N94	Peripheral neuritis/neuropathy
Infections / infection-related symptoms					
General symptoms		***Eye symptoms/diseases***		***Mouth symptoms/diseases***	
A02	Chills	F02	Red eye	D19	Teeth/gum symptom/complaint
A03	Fever	F70	Conjuctivitis infectious	D20	Mouth/tongue/lip symptom complaint
A04	Weakness/tiredness general	F72	Blepharitis/stye/chalazion		Teeth/gum disease
A05	Feeling ill	F73	Eye infection/inflammation other	D82	Mouth/tongue/lip disease
A29	General symptoms/compaint other			D83	

table continues

Viral and bacterial infections		*Ear, nose and throat symptoms/diseases*		*Skin symptoms/diseases*	
A70	Generalized tuberculosis (excluding tuberculosis lungs)	H70	Otitis externa	S10	Warts
		H71	Acute otitis media/myringitis	S11	Infected finger/toe
A71	Measles	H72	Serous otitis media	S70	Boil/carbuncle
A72	Chickenpox	H73	Eustachian salpingitis	S71	Local skin infection local
A73	Malaria	H74	Chronic otitis media	S72	Scabies/other acariasis
A74	Rubella	R09	Sinus symptom/complaint	S73	Pediculosis/skin infestation other
A75	Infectious mononucleosis	R21	Throat symptom/complaint	S74	Dermatophytosis
A76	Viral exanthem other	R22	Tonsils symptom/complaint	S75	Moniliasis/candidiasis skin
A77	Viral disease other/NOS	R73	Boil/abscess nose	S76	Skin infection other
A78	Infectious disease other/NOS	R74	Upper respiratory infection acute	S84	Impetigo
A92	Toxoplasmosis	R75	Sinusitis acute/chronic	S90	Pityriasis rosea
B02	Lymph gland(s) enlarged/painful	R76	Tonsillitis acute	S95	Molluscum contagiosum
B03	Symptoms/complaint lymph glands/immune other	R77	Laryngitis/tracheitis acute		
		R90	Hypetrophy tonsils/adenoid		
B70	Lymphadenitis acute	*Lung symptoms/diseases*		*Gastrointestinal symptoms/diseases*	
B71	Lymphadenitis non-specific	R25	Sputum/phlegm abnormal	D22	Worms/other parasites
D71	Mumps	R29	Respiratory symptom/complaint other	D70	Gastrointestinal infection
N72	Tetanus			D72	Viral hepatitis
R71	Whooping cough	R70	Tuberculosis lungs	D73	Gastroenteritis presumed infection
R72	Strep throat	R78	Acute bronchitis/bronchiolitis		Appendicitis
R80	Influenza (excluding pneumonia)	R81	Pneumonia	D88	Anal fissure/perianal abcess
S70	Herpes zoster	R82	Pleurisy/pleural effusion	D95	Cholecystitis/cholelithiasis
S71	Herpes simplex	R83	Respiratory infection other	D98	

table continues

Other		***Genital symptoms/diseases (female and male)***			
K70	Infection of circulatory system	X70	Syphilis female	Y70	Syphilis male
L70	Infections musculoskeletal system	X71	Gonorrhoea female	Y71	Gonorrhoea male
N71	Meningitis/encephalitis	X72	Genital candidiasis female	Y72	Genital herpes male
N73	Neurological infection other	X73	Genital trichomoniasis female	Y73	Prostatits/seminal vesiculitis
Urinary tract diseases		X74	Pelvic inflammatory disease	Y74	Orchitis/epididymitis
		X84	Vaginitis/vulvitis NOS	Y75	Balanitis
U70	Pyelonephritis/pyelitis	X85	Cervical disease NOS	Y76	Condylomata acuminata male
U71	Cystitis/urinary infection other	X90	Genital herpes female		
U72	Urethritis	X91	Condylomata acuminata female		
Rheumatoid arthritis-related comorbidities					
A12	Allergy/allergic reaction NOS	D85	Duodenal ulcer	P17	Tabacco abuse
B80	Iron deficiency anaemia	D86	Peptic ulcer other	P19	Drug abuse
B81	Anaemia, Vitamin B12/folate deficiency	D87	Stomach function disorder	P76	Depressive disorder
B82	Anaemia other/unspecified	F71	Conjunctivitis allergic	R02	Shortness of breath/dyspnoea
D01	Abdominal pain/cramps general	K75	Acute myocardial infarction	R03	Wheezing
D03	Abdominal pain epigastric	K78	Atrial fibrillation/flutter	R05	Cough
D04	Dyspepsia/indigestion	K79	Paroxysmal tachycardia	S97	Allergic rhinitis
D06	Abdominal pain localized other	K85	Elevated blood pressure	T15	Urticaria
D08	Flatulence/gas/belching	K89	Transient cerebral ischaemia	T81	Thyroid nodule/swelling
D09	Nausia	K93	Pulmonary embolism	T83	Obesity
D11	Diarrhoea	K94	Phlebitis/thrombophlebitis	T85	Overweight
D12	Constipation	P03	Feeling depressed	Z01	Poverty/financial problems
D18	Change faeces/bowel movements	P15	Chronic alcohol abuse	Z05	Work problem
D25	Abdominal distension	P16	Acute alcohol abuse	Z06	Unemployment problem

table continues

Chronic diseases					
B90	HIV-infection/aids	K91	Atherosclerosis/peripheral vascular disease	R96	Asthma
D92	Diverticular disease	L89	Osteoarthrosis of hip	S91	Psoriasis (with or without arthropathy)
D94	Chronic enteritis/ulcerative colitis	L90	Osteoarthrosis of knee	T82	Goitre
K74	Ischaemic heart disease with angina	L91	Osteoarthrosis other	T86	Hypothyreoidism/myxoedema
K76	Ischaemic heart disease without angina	L95	Osteoporosis	T90	Diabetes mellitus
K77	Heart failure	N70	Poliomyelitis	T92	Gout
K86	Hypertension uncomplicated	P72	Schizophrenia	U88	Glomerulonephritis/nephrosis
K87	Hypertension complicated	R91	Chronic bronchitis/bronchiectasis		
K90	Stroke/cerebrovascular accident	R95	Chronic obstructive pulmonary disease		Note: NOS = not otherwise specified

Supplementary Table 2. Univariate logistic regression analysis of the relation of individual ICPC-codes with IA development

ICPC	Description	Group	OR	CI	p-value
L20	Joint symptom/complaint NOS	Musculoskeletal	8.1	5.8 - 11.3	<0.01
L97	Chronic internal derangement knee	Musculoskeletal	5.9	1.6 - 21.8	<0.01
L11	Wrist symptom/complaint	Musculoskeletal	4.9	3.2 - 7.5	<0.01
NA	Other infectious symtoms	Infections	4.9	1.5 - 15.7	<0.01
L12	Hand/finger symptom/complaint	Musculoskeletal	4.0	3.1 - 5.1	<0.01
S91	Psoriasis	Chronic disease	3.7	2.5 - 5.4	<0.01
D94	Chronic enteritis/ulcerative colitis	Chronic disease	3.5	1.9 - 6.4	<0.01
T92	Gout	Chronic disease	3.5	2.6 - 4.7	<0.01
L29	Symptom/complaint musculoskeletal other	Musculoskeletal	2.9	1.9 - 4.4	<0.01
N93	Carpal tunnel syndrome	Musculoskeletal	2.7	1.9 - 4.0	<0.01
L92	Shoulder syndrome	Musculoskeletal	2.6	2.0 - 3.4	<0.01
L91	Osteoarthrosis other	Chronic disease	2.6	1.9 - 3.5	<0.01
L19	Muscle symptom/complaint NOS	Musculoskeletal	2.5	1.6 - 4.0	<0.01
B80	Iron deficiency anaemia	RA-related diseases	2.4	1.6 - 3.6	<0.01
B81	Anaemia, Vitamin B12/folate deficiency	RA-related diseases	2.4	1.5 - 3.6	<0.01
K94	Phlebitis/thrombophlebitis	RA-related diseases	2.3	1.4 - 3.8	<0.01
L08	Shouder symptom/complaint	Musculoskeletal	2.2	1.8 - 2.8	<0.01
L15	Knee symptom/complaint	Musculoskeletal	2.2	1.8 - 2.8	<0.01
L17	Foot/toe symptom/complaint	Musculoskeletal	2.2	1.8 - 2.7	<0.01
L18	Muscle pain	Musculoskeletal	2.2	1.7 - 3.0	<0.01
A01	Pain general/multiple sites	Musculoskeletal	2.2	1.4 - 3.5	<0.01
N94	Peripheral neuritis/neuropathy	Musculoskeletal	2.1	1.4 - 2.9	<0.01
T83	Overweight	RA-related diseases	2.1	1.04 - 4.3	0.04*

table continues

ICPC	Description	Group	OR	CI	p-value
L16	Ankle symptom/complaint	Musculoskeletal	2.0	1.2 - 3.4	<0.01
L01	Neck syptom/complaint	Musculoskeletal	1.9	1.5 - 2.4	<0.01
L02	Back symptom/complaint	Musculoskeletal	1.9	1.5 - 2.3	<0.01
L89	Osteoarthrosis of hip	Chronic disease	1.9	1.3 - 2.7	<0.01
L09	Arm symptom/complaint	Musculoskeletal	1.9	1.2 - 3.0	<0.01
L04	Chest symptom/complaint	Musculoskeletal	1.8	1.4 - 2.3	<0.01
L13	Hip symptom/complaint	Musculoskeletal	1.8	1.3 - 2.5	<0.01
A80	Trauma/injury NOS	Musculoskeletal	1.8	1.1 - 2.7	0.01
L90	Osteoarthrosis of knee	Chronic disease	1.7	1.3 - 2.3	<0.01
NA	Urinary tract symptoms	Infections	1.6	1.3 - 1.8	<0.01
D12	Constipation	RA-related diseases	1.6	1.2 - 2.0	<0.01
NA	Viral and bacterial symptoms	Infections	1.6	1.2 - 2.1	<0.01
T86	Hypothyreoidism/myxoedema	Chronic disease	1.6	1.2 - 2.0	<0.01
NA	Genital problems	Infections	1.6	1.2 - 2.1	<0.01
L95	Osteoporosis	Chronic disease	1.5	1.1 - 2.0	<0.01
K90	Stroke/cerebrovascular accident	Chronic disease	1.5	1.1 - 2.0	0.02
D11	Diarrhoea	RA-related diseases	1.5	1.03 - 2.3	0.04*
NA	General symptoms	Infections	1.4	1.2 - 1.7	<0.01
NA	Skin symptoms	Infections	1.4	1.2 - 1.7	<0.01
R05	Cough	RA-related diseases	1.4	1.2 - 1.7	<0.01
L03	Low back symptom/complaint	Musculoskeletal	1.4	1.1 - 1.7	<0.01
NA	Lung symptoms	Infections	1.4	1.1 - 1.7	<0.01
D06	Abdominal pain localized other	RA-related diseases	1.4	1.1 - 1.9	<0.01
R02	Shortness of breath/dyspnoea	RA-related diseases	1.4	1.04 - 2.0	0.03*
NA	Mouth symptoms	Infections	1.4	1.02 - 1.9	0.03*
K74	Ischaemic heart disease w. angina	Chronic disease	1.4	1.01 - 1.9	0.04*
K86	Hypertension uncomplicated	Chronic disease	1.3	1.1 - 1.5	<0.01
R96	Asthma	Chronic disease	1.3	1.03 - 1.6	0.03*

table continues

ICPC	Description	Group	OR	CI	p-value
K78	Atrial fibrillation/flutter	RA-related diseases	1.3	1.02 - 1.8	0.04*
K87	Hypertension complicated	Chronic disease	1.3	1.01-1.8	0.04*
L14	Leg/thigh symptom/complaint	Musculoskeletal	1.3	1.01 - 1.7	0.04*
NA	Ear, nose and throat symptoms	Infections	1.2	1.08 - 1.4	<0.01
T90	Diabetes mellitus	Chronic disease	1.2	1.1 - 1.5	<0.01

*These variables lost their significance after multiple testing correction using false positive rate control

Abbreviations: OR: odds ratio; CI: 95% confidence interval; NA: not applicable; NOS: not otherwise specified; IA: inflammatory arthritis

Supplementary Table 3. Multivariate logistic regression analysis of the relation of individual ICPC-codes with IA development (N=2314 cases and N=4541 controls)

ICPC	Description	Group	OR	CI	p-value	Obs*
L20	Joint symptom/complaint NOS	Musculoskeletal	7.9	5.5 - 11.1	<0.01	170/44
L97	Chronic internal derangement knee	Musculoskeletal	5.0	1.3 - 19.5	0.02	9/3
L11	Wrist symptom/complaint	Musculoskeletal	3.8	2.4 - 6.1	<0.01	73/30
S91	Psoriasis	Chronic diseases	3.8	2.5 - 5.8	<0.01	71/39
L12	Hand/finger symptom/complaint	Musculoskeletal	3.3	2.5 - 4.4	<0.01	179/94
D94	Chronic enteritis/ulcerative colitis	Chronic diseases	3.0	1.6 - 5.6	<0.01	30/17
T92	Gout	Chronic diseases	2.8	2.0 - 3.9	<0.01	119/69
L92	Shoulder syndrome	Musculoskeletal	2.2	1.6 - 2.9	<0.01	137/106
B80	Iron deficiency anaemia	RA-related diseases	2.1	1.4 - 2.7	<0.01	56/46
N93	Carpal tunnel syndrome	Musculoskeletal	2.0	1.3 - 3.0	<0.01	66/48
L15	Knee symptom/complaint	Musculoskeletal	1.9	1.5 - 2.4	<0.01	177/162
L91	Osteoarthrosis other	Chronic diseases	1.9	1.4 - 2.7	<0.01	101/78
B81	Anaemia, Vitamin B12/folate deficiency	RA-related	1.9	1.2 - 3.1	0.01	45/38

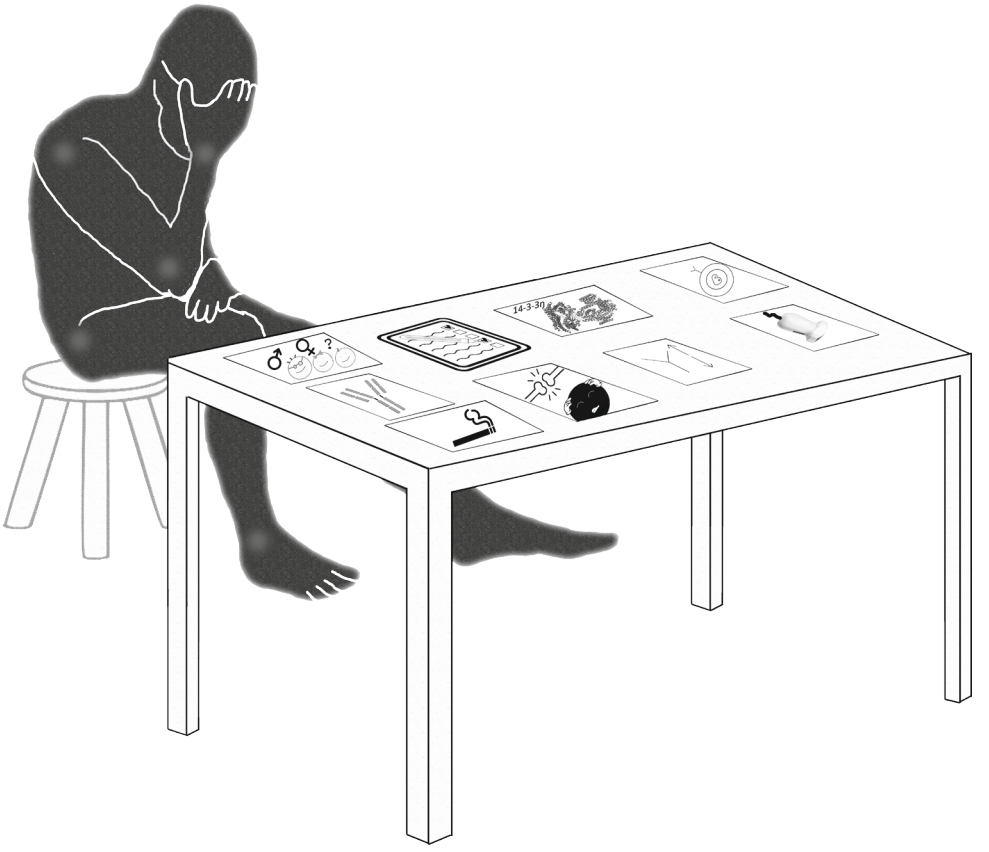
table continues

ICPC	Description	Group	OR	CI	p-value	Obs*
L29	Symptom/complaint musculoskeletal other	Musculoskeletal	1.8	1.1 - 2.9	0.01	52/36
K94	Phlebitis/thrombophlebitis	RA-related	1.8	1.03 - 3.2	0.04	31/27
L02	Back symptom/complaint	Musculoskeletal	1.7	1.3 - 2.1	<0.01	155/169
L08	Shouder symptom/complaint	Musculoskeletal	1.7	1.3 - 2.2	<0.01	188/173
L17	Foot/toe symptom/complaint	Musculoskeletal	1.7	1.3 - 2.2	<0.01	175/163
L18	Muscle pain	Musculoskeletal	1.7	1.3 - 2.4	<0.01	102/92
L04	Chest symptom/complaint	Musculoskeletal	1.6	1.3 - 2.1	<0.01	121/136
L89	Osteoarthrosis of hip	Chronic diseases	1.6	1.1 - 2.4	0.02	56/60
N94	Peripheral neuritis/neuropathy	Musculoskeletal	1.6	1.1 - 2.3	0.02	62/60
L01	Neck syptom/complaint	Musculoskeletal	1.5	1.1 - 2.0	<0.01	116/124
NA	Genital problems	Infections	1.5	1.1 - 2.1	<0.01	88/111
L13	Hip symptom/complaint	Musculoskeletal	1.5	1.04 - 2.1	0.03	79/86
NA	Urinary tract symptoms	Infections	1.4	1.1 - 1.6	<0.01	272/359
NA	Viral and bacterial symptoms	Infections	1.4	1.05 - 1.9	0.02	99/123
T86	Hypothyreoidism/myxoedema	Chronic diseases	1.4	1.04 - 1.8	0.03	105/134
K86	Hypertension uncomplicated	Chronic diseases	1.2	1.1 - 1.4	<0.01	516/827
R05	Cough	RA-related	1.2	1.0 - 1.5	<0.01	205/286
D09	Nausia	RA-related	0.4	0.2 - 0.8	<0.01	17/50
D08	Flatulence/gas/belching	RA-related	0.06	0.01 - 0.6	0.048	1/12
NA	Age	NA	1.0	0.99 - 1.0	0.500	NA
NA	Gender	NA	0.8	0.8 - 0.9	<0.01	NA

* Observations of number of patients (left: cases/right: controls) with that ICPC-code within the last 12 months Abbreviations: OR: odds ratio; CI: 95% confidence interval; NA: not applicable; NOS: not otherwise specified; IA: inflammatory arthritis

PART III

Markers for the development of rheumatoid arthritis



CHAPTER

7

A prospective cohort study of 14-3-3η in ACPA and/or RF-positive patients with arthralgia

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ABSTRACT

Background: 14-3-3η (eta) is a novel serum/plasma protein biomarker involved in the upregulation of inflammatory and joint damage factors. We analysed the association of 14-3-3η with the development of clinically apparent arthritis in a cohort of subjects with arthralgia and positivity for at least one serologic marker: rheumatoid factor (RF) or anti-citrullinated protein antibody (ACPA).

Methods*:* Measurement of 14-3-3η in plasma collected on entry into the cohort. For this study 144 subjects with a minimum of 2.5 (median and maximum 5) years follow-up were available. The relationship between presence and levels of 14-3-3η and development of arthritis was investigated.

Results*:* Arthritis occurred in 43 (30%) of the 144 subjects after a median of 15 months. 14-3-3η was detectable up to 5 years before onset of clinical arthritis and was present significantly more often (36% versus 14%; relative risk 2.5, 95% confidence interval 1.2-5.6; p=0.02) and at significantly higher levels (median 0.95 versus 0.28 ng/ml; p=0.02) in subjects developing arthritis compared with those who did not. 14-3-3η levels/positivity and ACPA, but not RF, were univariately associated with the development of arthritis while generalized linear model analysis with RF and ACPA as obligatory factors could not return an incremental benefit with 14-3-3η.

Conclusions*:* 14-3-3η was detectable prior to the onset of arthritis and was associated with arthritis development in arthralgia subjects preselected for positivity of RF or ACPA. Its power to predict onset of arthritis independent of ACPA and RF requires a new study in which patients are not preselected based on ACPA and/or RF-seropositivity.

BACKGROUND

The focus on the management of rheumatoid arthritis (RA) is increasingly towards early detection and treatment. Better prediction of the development of RA will potentially allow preventive interventions in these at-risk individuals. Recently, we published a prediction rule for the development of arthritis in rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPA) positive (seropositive) arthralgia patients[1]. Patients could be divided into high, intermediate or low risk categories quite accurately with a receiver operator characteristic (ROC) curve AUC (area under the curve) of 0.82 at 5 years. However, this is still inadequate for individual patient care - assuming the availability of a preventive intervention; therefore additional biomarkers to improve the predictive arthritis risk algorithm is required.

Several such potential biomarkers were recently described. Examples are anti-carbamylated protein antibodies (anti-Carp)[2], peptidyl arginine deiminase type 4 (anti-PAD-4)[3], and a high interferon gene score[4]. Two of these biomarkers were discovered within the same patient group as we will describe here[2 4]. Such biomarkers may help to improve prediction, but also offer new insights into the course of events leading to clinical arthritis.

Serum 14-3-3η (eta) is a novel protein biomarker showing potential in predicting radiographic deterioration in early and advanced RA[5 6]. 14-3-3 proteins belong to a family of seven isoforms known to bind to and regulate the biologic activity of various intracellular proteins[7]. Overexpression of 14-3-3 proteins is associated with worse outcomes in various diseases, such as cancers, neurodegenerative diseases and Creutzfeldt-Jakob's disease. The 14-3-3η isoform is expressed at higher levels in patients with arthritis compared with healthy individuals, which is thought to be related to 14-3-3η's direct ability to induce factors linked to inflammation and radiographic damage[8]. 14-3-3η has been shown to induce inflammatory factors such as interleukin (IL)-1 and -6, and is linked to the process of joint damage as it also induces factors such as receptor activator of nuclear factor kB ligand (RANK L) and matrix metalloproteinase (MMP) 1.

In this study, we analysed the association of baseline 14-3-3η with the development of clinically apparent arthritis in a cohort of subjects with arthralgia who were preselected based on being positive for at least one serologic marker: rheumatoid factor (RF) or anti-citrullinated protein antibody (ACPA).

PATIENTS AND METHODS

Study participants

From the Reade seropositive arthralgia cohort, the first 144 participants (with ≥30 months of follow-up or development of arthritis, included between 2004 and 2008) were used. This cohort was set up to determine clinical and serological risk factors for development of arthritis, and comprises subjects at-risk of arthritis, as defined by arthralgia (no history and no presence of clinically diagnosed arthritis at the time of their first physical examination

and no erosions on X-rays of hands and feet) and positivity for at least one serologic marker: ACPA or RF[9].

Study procedures

At baseline, all participants had clinical and demographic data collected (including visual analogue scale pain, morning stiffness, total painful and swollen joints) and provided a plasma sample through standard phlebotomy procedures. Enrolment was based on being positive for ACPA and/or RF. Plasma was stored at -20°C until blinded batch analyses were performed. Following baseline assessments, all participants were re-assessed at regular 12 month intervals over 5 years with emphasis on the development of clinical arthritis. An extra visit could be scheduled in case of arthritis development. Arthritis was defined based on the presence of at least one swollen joint on physical examination of 44 joints by a trained medical doctor (WB or LAS), who was aware of the status of ACPA and RF in the patient. In case of (uncertain) arthritis according to the first observer, the final judgment on presence or absence of arthritis was determined by a senior rheumatologist, who was unaware of the serostatus in the patient (DS). The study was approved by the Ethics Committee of Slotervaart Hospital and Reade, Amsterdam, The Netherlands, and written informed consent was obtained from all study participants.

Detection of biochemical markers

Baseline plasma was assessed for 14-3-3η levels using the quantitative 14-3-3η enzyme-linked immunosorbent assay (ELISA, Augurex Life Sciences Corp, Vancouver, Canada). Positivity for 14-3-3η was defined as ≥0.19 ng/ml based on the manufacturer's recommended cut-off, and at 2 times and 4 times this cut-off. The development, validation and calibration of the assay are detailed in a recent publication[6]. ACPA was measured by an anti-CCP2 ELISA (Axis Shield, Dundee, UK) and IgM-RF by an in-house ELISA as described previously[10]. The cut-off level for ACPA positivity was set at ≥5 arbitrary units/ml (AU/ml), according to the manufacturer's instructions. The cut-off level for IgM-RF positivity was set at ≥30 international units/ml (IU/ml).

Statistical Methods

The primary outcome chosen was arthritis, not rheumatoid arthritis to prevent circularity, as ACPA and RF are present in the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) RA criteria and 14-3-3η is not. Continuous, normally distributed data were presented as mean (standard deviation) and two-tailed t tests were used to establish whether significant differences existed between groups. Non-normally distributed data were presented as median (interquartile range) and analysed by Mann-Whitney U tests. The Fisher's exact test was used to identify if positivity for any of the serologic variables investigated (ACPA, RF, and 14-3-3η) was significantly associated with arthritis development over 5 years. Spearman's rank correlation coefficients expressed the relationship between 14-3-3η and the other serological markers ACPA and RF. Cox-proportional hazards survival analysis tested whether 14-3-3η can predict time to arthritis development.

Generalized linear models (GLM) assessed whether 14-3-3η was independently associated with the development of arthritis within 5 years. We used GLM with binomial outcome and log-link function, rather than standard logistic regression, because of the opportunity to describe relative risks (RR) instead of odds ratios, as this is a more proper association measure for describing results from prospective cohort studies. Since enrolment in the study implied that a subject was either ACPA or RF positive (or both) and no data was obtained in a group negative on both ACPA and RF, we jointly corrected for ACPA and RF status using a categorical variable distinguishing the 3 groups: (1) only RF positive, (2) only ACPA positive, (3) both RF and ACPA positive. Thereafter we created a variable containing 14-3-3η at different cut-off points (as mentioned above). In the GLM we first put in the categorical variable, after which we added 14-3-3η. The generated p values for 14-3-3η can then be interpreted as follows; if significance is found then the 14-3-3η test adds predictive value to the ACPA and RF test in the case one or both of these tests are positive. Note that this significance will imply that the additive value is the same for all 3 categories. To test whether predictive performance of 14-3-3η depends on the outcome of the ACPA and RF test, we also performed interaction analysis (by adding the interaction between the categorical variable and 14-3-3η in multivariable analyses). This interaction analysis will reveal whether 14-3-3η has more predictive capacity in one of the 3 groups. All analyses were performed with SPSS version 21 (IBM Corp, Armonk, NY, USA).

RESULTS

Arthritis development

Forty-three out of a total of 144 subjects (30%) developed arthritis after a median of 15 months (Table 1). The median follow-up of subjects not developing arthritis was 60 months (minimum 30 months). Ninety-five percent of the subjects developing arthritis fulfilled the 2010 ACR/EULAR classification criteria for RA[11]. Of those, 28% fulfilled the criteria regardless of their ACPA and RF serostatus. Five subjects had erosions on their hands or feet X-rays at the time of arthritis diagnosis (out of 36 subjects with X-rays performed). Compared with the subjects not developing arthritis, those that did had significantly more morning stiffness and pain, higher ACPA levels and positivity, and higher 14-3-3η levels and positivity at baseline. Importantly, RF, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were not significantly different between the 2 groups. At baseline 29% of subjects used non-steroidal anti-inflammatory drugs (NSAIDs) and no patients received hydroxychloroquine. During the course of the study, 42% used NSAIDs at one or more time points, and 5% received hydroxychloroquine (of these patients 5 did not develop arthritis whilst 2 did). Notably, 31 subjects (22%) received 1-2 dexamethasone injections after baseline in a double-blind trial (which did not delay or prevent arthritis development)[9].

Serological biomarkers 14-3-3η, ACPA and RF

As represented in Table 1, median 14-3-3η expression levels at baseline were significantly higher in the 43 subjects who developed arthritis in comparison with 101 subjects that did not develop arthritis (median 0.95 vs 0.28, $p<0.01$). Table 1 together with Figure 1 demonstrate that the prevalence of 14-3-3η positivity at baseline was significantly greater

in those patients that developed arthritis in comparison with those that did not at the different cut-off points (86% vs 64%, p<0.01; 58% vs 40 %, p=0.04; 51% vs 24%, p<0.01 for cut-offs 0.19, 0.4 and 0,8 respectively). Also, the distribution of positivity for ACPA, RF and 14-3-3η and the different combinations between those that developed arthritis and those who did not is outlined in Figure 1. It shows that subjects developing arthritis were either in the subgroup of ACPA/14-3-3η positives (30%) or ACPA/RF/14-3-3η positives (52%). Spearman's rank sum revealed that levels of 14-3-3η were moderately correlated with those of RF and ACPA (0.30 and 0.31, respectively; p<0.01). Performance characteristics of 14-3-3η were as follows for the 0.19 cut-off point (manufacturer's recommended cut-off): sensitivity 36%, specificity 86%, positive predictive value 86% and negative predictive value 36%. Univariate GLM analysis indicated that baseline 14-3-3η positivity significantly predicted arthritis development delivering RRs of 2.5 (p=0.02), 1.7 (p=0.04) and 2.2 (p<0.01) at the cut-off points ≥0.19, ≥0.40 and ≥0.80 ng/ml, respectively (Table 2, upper part). GLM evaluating 14-3-3η levels further revealed 14-3-3η's association with the arthritis outcome, with an RR of 1.04 (p=0.01). As previously reported from this cohort, ACPA positivity had a strong association with arthritis development (RR 10.9, p<0.01, measured univariately), but RF positivity did not. In multivariable GLM (Table 2, lower part) 14-3-3η levels and positivity at all cut-off points were corrected for the autoantibody status of ACPA and/or RF. Since we used a categorical variable for ACPA and/or RF presence, the generated p values for 14-3-3η can be interpreted as predictive capacity of 14-3-3η in the case one or both of these ACPA/RF tests are positive. In this situation, neither 14-3-3η levels nor positivity at any cut-off point added value to the prediction of arthritis development. In the interaction analyses the added value of a positive 14-3-3η test did not differ between subjects that were only RF positive, only ACPA positive or those who were positive for both tests. No significant relation between either 14-3-3η positivity or levels and time of arthritis onset could be found in the Cox proportional hazards model (data not shown). Subgroup analysis of subjects with certain combinations of biomarkers, for example 14-3-3η positivity in ACPA negative versus positive subjects, was not feasible due to small subgroups.

Table 1. Baseline characteristics of study participants

Variable	Total group (n=144)	Arthritis (n=43)	No arthritis (n=101)	p value
Time until end of follow-up (censoring or arthritis; months)	60 (1-60)	15 (0-60)	60 (30-60)	<0.01
Age (years)*	55 (11)	54 (11)	56 (12)	NS
Males (%)	23	28	21	NS
Disease activity				
Tender joint count 53	0 (0-5)	0 (0-2)	0 (0-5)	NS
Visual analogue scale pain	29 (0-100)	35 (0-100)	26 (0-98)	NS
Use of NSAIDs (%)	29	35	26	NS
ESR (mm/hour)	11 (0-34)	11 (0-34)	11 (1-31)	NS

table continues

Variable	Total group (n=144)	Arthritis (n=43)	No arthritis (n=101)	p value
CRP (mg/l)	2 (0-47)	2 (0-47)	3 (0-27)	NS
Fulfilment of 2010 ACR/EULAR classification criteria for RA (%)	NA	95	0	NA
14-3-3η results				
Level (ng/ml)	0.35 (0.03-20)	0.95 (0.12-20)	0.28 (0.03-20)	<0.01
≥ 0.19 ng/ml (%)	71	86	64	<0.01
≥ 0.40 ng/ml (%)	45	58	40	0.04
≥ 0.80 ng/ml (%)	33	51	24	<0.01
RF results				
Level (IU/ml)	38 (1-1192)	31 (1-383)	40 (1-1192)	NS
positivity (%)	63	61	63	NS
ACPA results				
Level (AU/ml)	108 (0-9860)	455 (0-8710)	59 (0-9860)	<0.01
positivity (%)	65	95	53	<0.01

* Mean (SD), all other continuous variables mentioned as median (min-max).
Abbreviations: ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RA: rheumatoid arthritis; NSAIDs: non-steroidal anti-inflammatory drugs; AU/ml: arbitrary units/ml; IU/ml: international units/ml; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibodies; NS: not significant (p-value ≥ 0.05); NA: not applicable.

Table 2. Univariate and multivariable association of 14-3-3η, ACPA and RF with arthritis development

Univariate logistic regression		
Variable	RR (95% CI)	p value
14-3-3η		
cut-off ≥ 0.19	2.5 (1.2-5.6)	0.02
cut-off ≥ 0.40	1.7 (1.0-2.8)	0.04
cut-off ≥ 0.80	2.2 (1.3-3.5)	< 0.01
levels	1.04 (1.01-1.07)	0.01
Multivariable logistic regression		
Variable		RR (95% CI)
Categorical variable:		
RF		reference
ACPA		7.9 (1.9-32.4)
RF and ACPA		15.0 (3.8-59.7)

table continues

Variable	RR (95% CI)	p value
Adding 14-3-3η to the above categorical variable		
14-3-3η ≥ 0.19		1.6 (0.7-3.5)*
14-3-3η ≥ 0.40		1.2 (0.7-1.8)
14-3-3η ≥ 0.80		1.3 (0.8-2.1)
14-3-3η levels		1.01 (0.98-1.04)**

Abbreviations: RR: relative risk; CI: confidence interval

***** *Interpretation of the relative risk, example: subjects that are 14-3-3η positive at the 0.19 cut-off point have a 1.6 times higher risk of developing arthritis, given the knowledge that these subjects must be either RF or ACPA positive.*

*** For continuous variables the relative risk conveys the higher risk of developing arthritis per ascending unit of the independent variable, in this case 14-3-3η.*

DISCUSSION

This study presents data that 14-3-3η is present in the pre-clinical phase of arthritis development, since there was a greater proportion of positivity of this marker at study entry together with higher expression in a preselected cohort of ACPA and/or RF positive arthralgia subjects who developed arthritis, compared with those who did not. This may be related to 14-3-3η's ability to induce various inflammatory and joint degradative factors[6 8].

Since 14-3-3η is an inflammatory mediator, the mechanism through which it is related to the development of arthritis may be different from that of autoantibodies such as ACPA and RF, whose levels tend to remain static or unchanged over the course of one's disease. In this regard, a possible link of 14-3-3η with non-specific measures of inflammation, such as ESR and CRP, might be revealing. However, measurements of ESR and CRP at baseline were related neither to development of arthritis nor to 14-3-3η positivity in this cohort (data not shown). Another difference with autoantibodies might be the dynamic nature of serum 14-3-3η, which was supported by a study in first degree relatives of Indigenous North Americans with RA[12]. This study population was not suitable for serial measurements since half of the patients developed arthritis shortly after inclusion and therefore missed a secondary measurement of 14-3-3η.

In this study, although 14-3-3η was associated with the development of arthritis, baseline 14-3-3η levels and positivity at 3 cut-off points did not add predictive value to the combination of ACPA and RF. This is most likely influenced by both the pre-selection method for this cohort, the ascertainment of arthritis, as well as the dynamic nature of 14-3-3η. In particular, the blinded confirmatory rheumatologist reviewed only those suspected of developing arthritis, and not all 144 subjects. Since the unblinded physician was making the initial assessment of arthritis development, if a bias did exist from their knowledge of ACPA and RF status, the blinded confirmatory physician would have reviewed a predominance of, say, ACPA+ subjects and therefore identified more arthritis among those who were ACPA+.

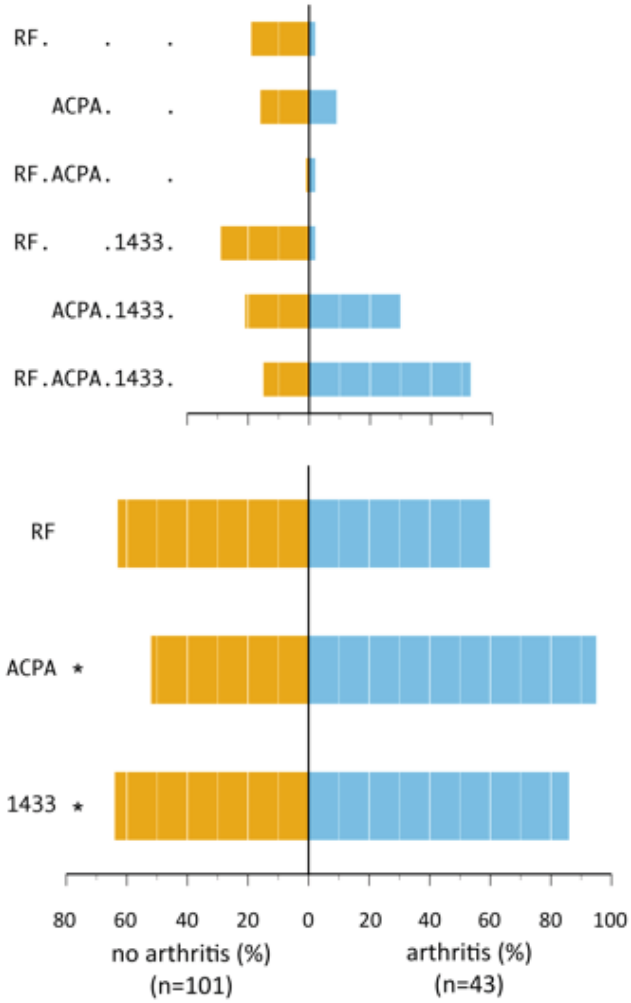

Figure 1. Distribution of positivity for ACPA, RF and 14-3-3η in a cohort of arthralgia patients Top panel: Different combinations of positive markers between the subjects developing arthritis versus those who do not; expressed as proportions. Bottom panel: Percentage of positivity of each marker in the subjects developing arthritis versus those who do not (* means a statistically significant difference with a p value <0.01).
Abbreviations: RF: rheumatoid factor; ACPA: anti-citrullinated protein antibodies; 1433; 14-3-3η protein (cut-off ≥0.19 ng/ml)

For clinical practice it would be very useful if 14-3-3η positivity could enhance the prediction of (rheumatoid) arthritis when combined with ACPA and RF. One such study that

would enable such an analysis comes from a cohort of subjects based on clinically suspect arthralgia at-risk for RA rather than on the basis of positive serology results. In addition to this, a prospective cohort recruited based on the presence of either of the 3 markers ACPA, RF or 14-3-3η may avoid any underestimation of the predictive capacity of 14-3-3η[13]. Another suggestion would be to use a design which includes serial measurements of 14-3-3η. The OMERACT working group has recommended this design in guidelines to study soluble biomarkers, aimed at clinical validation of their predictive capacity, particularly for prognostic end-points[14]. The major limitation of baseline assessment alone has been repeatedly emphasized, particularly for responsive biomarkers such as 14-3-3η, which could vary considerably over the course of disease, and also with therapeutic intervention. This is highlighted in a recent publication describing clinical validation of IL-6 as a predictor of an event where longitudinal, but not baseline assessment alone, was predictive of structural damage in RA[15]. The publication is about progression of radiographic damage, but it applies to other end-points as well. However, a single assessment does conform with the clinical situation where a decision is often made to follow the patient or not.

Another limitation of this study was that the primary outcome could not be rheumatoid arthritis, as ACPA and RF are part of the 2010 ACR/EULAR classification criteria and 14-3-3η is not. We explored alternative outcomes such as subjects fulfilling the ACR/EULAR criteria regardless of serostatus and the development of erosions, but not enough subjects were positive for either to allow meaningful analysis.

CONCLUSIONS

In conclusion, we have shown that 14-3-3η is often present in arthralgia subjects positive for ACPA and/or RF prior to the development of arthritis, and was associated with the development of arthritis. In this cohort of subjects preselected for ACPA and/or RF-positivity the added predictive value of 14-3-3η, both levels and different cut-off points, could not be established. Further studies are warranted to assess the combined utility of these 3 markers in predicting the development of arthritis.

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CHAPTER

8

Can increase of autoantibody levels predict arthritis in arthralgia patients?

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Abstract

Objective: An at risk phase of rheumatoid arthritis (RA) has been recognized in which autoantibodies such as anti-citrullinated protein antibodies (ACPA) and IgM rheumatoid factor (IgM-RF) can occur, often accompanied by arthralgia. Blood donor cohorts have shown a rise in autoantibody levels 1-3 years preceding diagnosis of RA. The present study was undertaken to assess the clinical value of individual prediction models based on trends in autoantibody levels over time.

Methods: 263 patients from an ongoing prospective cohort of ACPA and/or IgM-RF positive arthralgia patients, for whom repeated measurements of ACPA/IgM-RF were available, were followed for arthritis development. We fitted joint models of ACPA/IgM-RF levels over time combined with time-to-event data for arthritis.

Results: Amongst those that were observed to develop arthritis (N=69, 26%) 50% did so within 22 months. We used 983 measurements of ACPA/IgM-RF, with a median of 4 (range 2-8) per person. Time-updated ACPA was significantly associated with the development of arthritis with a hazard ratio (HR) of 2,8 (95%CI: 2.0-3.9, $p<0.001$, AUC 0.836). However, the added value of repeated measurements in the prediction model over baseline values alone was fairly small (AUC 0.027). IgM-RF levels did not have predictive value.

Conclusion*:* In seropositive arthralgia patients, ACPA was a good predictor of arthritis development, but the inclusion of time-updated measurements has limited additional predictive value over a baseline measurement. IgM-RF measurement had no predictive value for development of arthritis.

Clinically apparent rheumatoid arthritis (RA) may be preceded by a phase of autoimmunity with or without arthralgia[1]. The relevance of autoantibodies in the preclinical and early phase of RA is illustrated by the fact that prediction rules for the development of RA have included them[1], and that they are part of the 2010 American College of Rheumatology/ European League Against Rheumatism classification criteria for the classification of RA[2]. The best studied autoantibody systems preceding the diagnosis of RA are anti-citrullinated protein antibodies (ACPA) and IgM rheumatoid factor (IgM-RF, hereafter RF). Both the extent to which autoantibodies are found and the associated risk of future RA depend on which population is investigated.

Retrospective studies have included multiple measurements of autoantibodies before the diagnosis of RA[3 4]. ACPA could be detected up to 14 years and RF up to 11 years before symptom onset in a Dutch blood donor cohort[3]. Also, the closer the individual came to the diagnosis the more positivity for either one or the combination was found, together with increasing levels. This was confirmed for ACPA and IgA-RF in a Swedish blood donor cohort, in which the risk of developing arthritis based on autoantibody levels was higher with measurements within 1.5 years before diagnosis[4]. In the abovementioned blood donor cohorts as well as an American cohort, epitope spreading and increase in levels of various ACPA were noted in the last 2-3 years before diagnosis of RA[5-7]. However, to our knowledge no individual prediction models using autoantibody levels over time have been developed yet.

In this prospective study we investigated whether ACPA and RF levels and their change over time within individuals have added value for prediction of future arthritis in ACPA and/or RF positive individuals without arthritis.

PATIENTS AND METHODS

Study population. Between 2004 and 2012, patients with a positive ACPA and/or RF status and (a history of) arthralgia were recruited at rheumatology outpatient clinics in the Amsterdam area of the Netherlands[8]. Patients with past arthritis or arthritis at baseline (defined as 1 or more swollen joints as reported by two independent investigators), age <18 years and >70 years, previous treatment with a disease modifying antirheumatic drug or recent glucocorticoid treatment, systemic autoimmune disease, systemic infections, lymphoproliferative disorders or recent radiotherapy were excluded from the cohort. To enter the present study, patients had to have a follow-up of at least 6 months and be free of arthritis within this time period. Also, they needed to have their autoantibody status measured at least twice with the same antibody test since we correct the auto-antibody levels for baseline values and thus need at least one other measurement per person. In total, 104 out of 441 thus selected patients were excluded due to a follow-up of less than 6 months or the participation in a trial of dexamethasone which had effect on the autoantibody levels during the first half year[9]. Another 61 had only one measurement of the antibodies and 13 had missing values in their baseline characteristics, leaving 263 ACPA and/or RF positive arthralgia patients available for analysis (flow chart in supplementary

material). Written informed consent was obtained from all patients and the study was approved by the Slotervaart hospitals ethics committee.

Procedures.
Cohort visits including history, physical examination and laboratory investigations were performed semiannually the first year and yearly thereafter. Patients were followed for 5 years or until arthritis occurred. Clinical arthritis development (in at least one joint) was confirmed by physical examination of 44 joints by a trained medical doctor (LAS or MBT) and confirmed by a senior rheumatologist (DS).

Laboratory investigations.
ACPA was measured as anti-cyclic citrullinated peptide antibodies (anti-CCP) by second-generation anti-CCP enzyme-linked immunosorbent assay (ELISA, Axis shield, Dundee, United Kingdom). The cut-off level for anti-CCP positivity was set according to the manufacturer's instructions at ≥5 arbitrary units/ml. IgM-RF levels were determined by in-house ELISA. It was calibrated with a national reference serum containing 200 IU/ml and was recorded as positive when values were ≥30 IU/ml on the basis of Receiver Operator Characteristic (ROC) [3].

Statistical analysis.
A 10log transformation of ACPA and RF levels was used. A multivariable Cox proportional hazards model including baseline ACPA and RF and relevant clinical covariates (baseline alcohol use, smoking, age, gender, positive family history for RA, visual analogue scale (VAS) pain, morning stiffness ≥1 hour, duration of joint pain before inclusion, intermittent joint pain yes or no, tender joint count of 53 joints) was constructed to analyze the association with arthritis development. This was in analogy to a prediction model constructed with the entire cohort[10]. We report hazard ratios for ACPA and RF together with 95% confidence intervals (CI) and p-values. Also, we calculated the cumulative/dynamic area under the curve (AUC) of this model to quantify the accuracy of the model in predicting RA within the first 3 years[11]. The prediction horizon of 3 years was chosen to be able to compare it with the AUC of the joint models (see further). To combine the longitudinally measured antibodies and their relation with arthritis development, we fitted joint models using the R package JM (available at the Comprehensive R Archive Network; CRAN)[12]. In this analysis, a linear mixed effects model of the development of ACPA and RF over time and a Cox-proportional hazards survival model for the event hazard were combined. In this way it is possible to estimate the risk on future arthritis, using baseline as well as longitudinal (time-updated) measurements of ACPA and RF. We made three types of joint models: 1. "Univariate": Including either time-updated ACPA or RF as predictor variables; 2. "Bivariate" Including time-updated ACPA or RF while controlling for the other autoantibody at baseline; 3. "Full models": Including ACPA or RF, while controlling for the other (10log transformed) autoantibody at baseline, and including all other relevant clinical variables (see earlier description of the Cox proportional hazard analyses). The individual estimated slopes of ACPA and RF were also included in the models to see if the slope gives additional information over the values at different timepoints. The uncertainty

in both the autoantibody values as well as the slope is taken into account in these models. The cumulative/dynamic AUC's of the conventional Cox proportional hazards model was compared to those of the time-updated (joint) models to evaluate the added value of using repeated measurements of antibodies for individual prediction of the outcome. For the AUC of the joint models also a prediction window of 3 years was used, hereby using time-updated ACPA and RF values in the first 2 years as predictors for the outcome at 5 years.

All statistical analyses were performed using the statistical software R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

A total of 983 antibody measurements were obtained from 263 patients, a median of 4 per person (range 2-8). The measurements were 3 to 60 months apart (median 12 months). Amongst those that were observed to develop arthritis (N=69, 26%) 50% did so within 22 months. Of these 69 patients, 87% fulfilled the 2010 ACR/EULAR classification criteria. Patients who did not develop arthritis had a median follow-up of 58 months. For thirteen patients one or more baseline characteristics used in the models were not available, and these patients were excluded from the current analyses. ACPA positive patients, patients with morning stiffness ≥1 hour or intermittent joint pain in the past, and patients currenly smoking were more likely to develop arthritis. All other patient characteristics were not significantly related to arthritis (see Table 1).

Table 1. Baseline characteristics of study participants

Baseline characteristics	Total group	Arthritis	No arthritis	p-value*
	(N=263)	(N=69)	(N=194)	
Age**	50 (12)	49 (11)	51 (12)	0.582
Gender (male)	62 (24%)	19 (28%)	43 (22%)	0.317
Tender joint count at physical examination	0 (0-2)	0 (0-4)	0 (0-2)	0.143
Visual analogue scale pain	30 (10-55)	40 (15-57)	30 (7-50)	0.465
Morning stiffness ≥ 1 hour	41 (16%)	16 (23%)	25 (13%)	0.022
Smoking (yes)	77 (29%)	26 (38%)	51 (26%)	0.025
Alcohol use (yes)	173 (66%)	39 (57%)	134 (69%)	0.113
Caucasian (yes)***	171 (65%) (out of N=230)	41 (59%) (out of N=58)	130 (67%) (out of N=172)	0.436
RA in family history (yes)	89 (34%)	29 (42%)	60 (31%)	0.169
Duration of symptoms before inclusion (months)	15 (9-36)	12 (6-36)	15 (9-36)	0.920
Symptoms in upper and lower extremities (yes)	122 (46%)	37 (54%)	85 (44%)	0.203

table continues

Baseline characteristics	Total group	Arthritis	No arthritis	p-value*
Intermittent joint symptoms in the pas (yes)	80 (30%)	35 (51%)	45 (23%)	<0.001
Number of ACPA and RF measurements**	4 (2-8)	4 (2-8)	3 (2-5)	<0.001
ACPA positive	166 (63%)	64 (93%)	102 (53%)	<0.001
RF positive	159 (60%)	37 (54%)	122 (63%)	0.283
ACPA and RF positive	62 (24%)	32 (46%)	30 (16%)	<0.001
ACPA levels in total group	9 (0-50)	29 (12-103)	6 (0-27)	0.003
RF levels in total group	40 (11-80)	30 (10-70)	43 (11-81)	0.529
ACPA levels amongst ACPA positives	28 (5-1000)	31 (7-1000)	22 (5-615)	0.441
RF levels amongst RF positives	70 (30-1192)	68 (30-881)	71 (30-1192)	0.666

** All p-values obtained from Cox proportional hazard analyses.*
*** Age as mean (SD) and number of ACPA and RF measurements as median (range), all other continuous variables are reported as median (25th and 75th percentiles).*
**** In this variable (merely informative, not used in the models) 33 missing values occurred, all other covariates were completely measured.*
Abbreviations: ACPA: anti-citrullinated protein antibodies; RA: rheumatoid arthritis; RF: rheumatoid factor; SD: standard deviation.

Autoanitbody status conversion
Forty-three patients changed status for ACPA and/or RF positivity during the study period. Of these, 14 became ACPA negative within the patients not developing arhritis (1 also RF negative), and 1 in the arthritis group (with a highest ACPA baseline level of 21 AU/ml). Fifteen versus 2 became RF negative in the non-arthritis and arthritis groups, respectively. Only one became ACPA positive, while already being RF positive, and did not develop arthritis (highest ACPA level 8 AU/ml). Of the last 10 patients that became RF positive, while already being ACPA positve, 5 developed arthritis. Additionally, 19 patients had a positive ACPA level under 3 times the Upper Limit of Normal (ULN) at baseline and subsequently converted to >3x ULN at any post-baseline time point, of which 11 developed arthritis. On the contrary, 8 had high level ACPA at baseline that dropped <3x ULN at any point, but still 4 developed arthritis.

Survival model
The hazard ratio (HR) for baseline ACPA was 2.6 in the full Cox proportional hazards survival model (95% CI 1.9-3.6; p-value <0.001). In this model RF was not significantly related to timing of arthritis development (HR 1.1, CI 0.7-1.7; p-value 0.708). The cumulative/dynamic AUC was 0.809. Baseline ACPA contributes highly to this AUC, since the AUC of the full model without inclusion of ACPA was only 0.721 and that of a model with baseline ACPA alone was 0.733 (data not shown).

Joint models

Estimates from all constructed joint models are reported in Table 2. The HR for ACPA alone in the joint model was 2.3 (CI 1.8-3.1; p-value <0.001; AUC 0.739) and for RF alone 1.0 (CI 0.6-1.5; p-value 0.968; AUC 0.633). For ACPA this should be interpreted as follows: time-updated ACPA levels (adjusted for ACPA at baseline) were associated with a 2.3-fold higher rate of developing arthritis over time. The full joint models including all relevant clinical variables showed HRs of 2.8 (CI 2.1-3.9; p-value <0.001) for ACPA and 1.2 (0.7-2.0; p-value 0.460) for RF. In the models the ACPA and RF slopes both did not show a significant association with the outcome arthritis.The AUC of the model with ACPA controlling for baseline RF and including all other relevant clinical variables and the ACPA slope was 0.836. A line graph of individual ACPA and RF development over time for patients who developed arthritis and those who did not is shown in Figure 1.

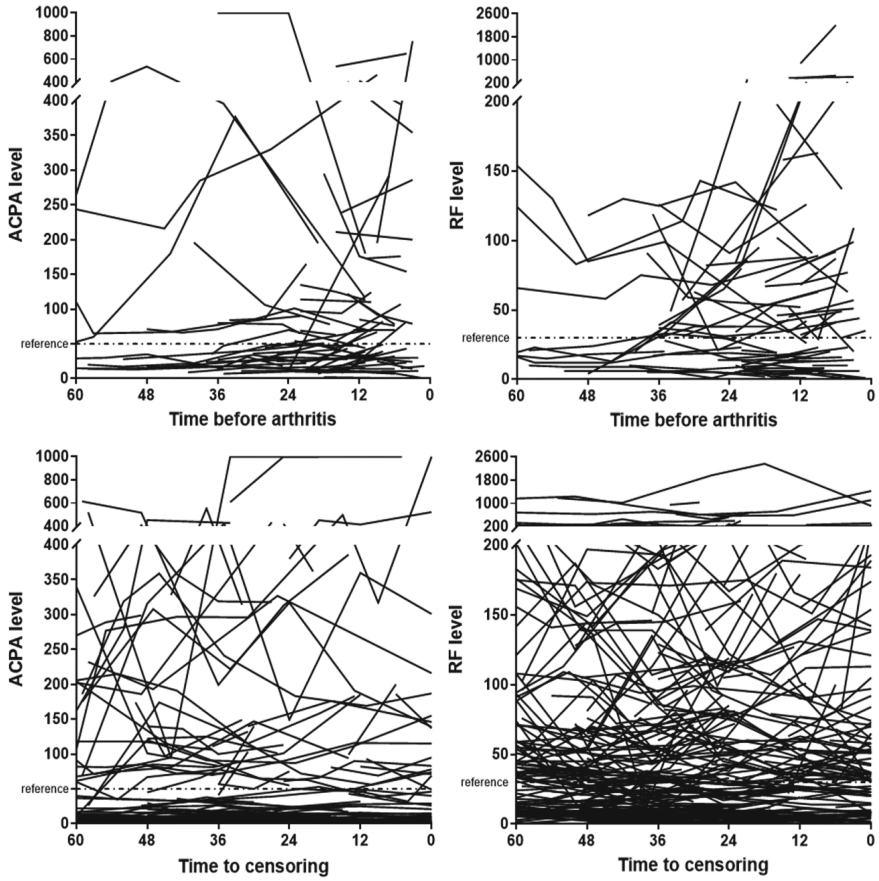

Figure 1. ACPA and RF levels according to time before arthritis (A and B); ACPA and RF levels according to time to censoring in patients without arthritis (C and D). Time in months

Table 2. Cox proportional hazard and joint models of ACPA and RF levels

Multivariable Cox proportional hazards model with the baseline ACPA and RF value*				
	Variables	HR (95% CI)	p-value	AUC
	ACPA	2.6 (1.9-3.6)	<0.001	0.809
	RF	1.1 (0.7-1.7)	0.708	
Joint models, using time-updated values of ACPA or RF**				
ACPA	Variables	HR (95% CI)	p-value	AUC
1.	Time updated ACPA alone	2.3 (1.7-3.1)	<0.001	0.739
2.	Time updated ACPA	2.4 (1.8-3.1)	<0.001	0.757
	RF (baseline value)	1.3 (0.8-2.1)	0.234	
3.	Time updated ACPA	2.8 (2.0-3.9)	<0.001	0.836
	ACPA slope (per year)	1.5 (0-10.000)	0.923	
	RF (baseline value)	1.3 (0.8-2.2)	0.351	
	Age (per decade)	1.1 (0.9-1.4)	0.361	
	Gender	0.9 (0.5-1.6)	0.646	
	Tender joint count 53	1.01 (0.95-1.07)	0.727	
	Visual analogue scale pain (per 10 mm)	1.01 (0.92-1.11)	0.830	
	Morning stiffness	2.6 (1.3-5.0)	0.006	
	Smoking	1.6 (0.9-2.9)	0.096	
	Alcohol use	0.5 (0.3-0.8)	0.009	
	Positive family history members	1.7 (1.0-2.9)	0.052	
	Duration of symptoms before inclusion (years)	1.04 (0.96-1.13)	0.323	
	Symptoms in upper and lower extremities (yes)	1.2 (0.7-2.0)	0.613	
	Intermittent joint pain (yes)	2.5 (1.5-4.3)	<0.001	
RF	Variables	HR (95% CI)	p-value	AUC
1.	Time updated RF alone**	0.9 (0.6-1.5)	0.732	0.633
2.	Time updated RF	1.3 (0.8-2.1)	0.276	0.757
	ACPA (baseline value)	2.3 (1.8-3.1)	<0.001	
3.	Time updated RF	1.3 (0.8-2.2)	0.287	0.795
	RF slope (per year)	5.1 (0-9000)	0.690	
	ACPA (baseline value)	2.7 (2.0-3.7)	<0.001	
	Age (per decade)	1.0 (0.9-1.4)	0.436	

table continues

Multivariable Cox proportional hazards model with the baseline ACPA and RF value*		
Gender	0.8 (0.4-1.6)	0.572
Tender joint count 53	0.995 (0.93-1.05)	0.877
Visual analogue scale pain (per 10 mm)	1.01 (0.92-1.12)	0.767
Morning stiffness	2.6 (1.4-5.1)	0.004
Smoking	1.8 (1.0-3.2)	0.042
Alcohol use	0.4 (0.2-0.8)	0.004
Positivity for having family members	2.0 (1.2-3.4)	0.013
Duration of joint pain before inclusion (years)	1.06 (0.98-1.15)	0.170
Symptoms in upper and lower extremities (yes)	1.2 (0.7-2.1)	0.520
Intermittent joint pain (yes)	2.5 (1.4-4.3)	0.001

** The multivariable model also contains clinical parameters (not shown here; baseline alcohol use, smoking, age, gender, positive family history for RA, visual analogue scale pain, morning stiffness ≥1 hour, duration of joint pain before inclusion, intermittent joint pain yes or no, tender joint count of 53 joints)*

*** ACPA and RF are 10logtransformed in all models (including the multivariable Cox model).*

Abbreviations: ACPA: anti-citrullinated protein antibodies; RF: rheumatoid factor; HR: hazard ratio; CI: confidence interval; AUC: area under the curve.

DISCUSSION

The observation of an increase of autoantibody levels starting approximately one to three years before clinical arthritis in blood donor chorts of preclinical RA suggests that this phenomenon might be useful for the prediction of future arthritis in autoantibody positive individuals[3 4]. Therefore, we studied autoantibody levels over time in a prospective cohort of ACPA and/or RF positive arthralgia patients. The results show that higher time-updated ACPA values were significantly associated with the development of arthritis, however, the same was observed for the baseline value. The added value of the time-updated ACPA levels (including clinical variables) was fairly small as compared to the model that only used baseline values (diffence in AUC of + 0.027 and no additional predictive value of the ACPA slopes in the joint model). Increase of RF levels at baseline and over time was not significantly associated with arthritis development in the Cox proportional hazards model and joint models respectively.

On the individual level, the patterns of autoantibody levels over time differed widely, regardless of the outcome: in both groups several subjects showed a pattern of either increasing, stable, decreasing or increasing/decreasing autoantibody levels. This appears to contradict the findings of studies that showed a rise in autoantibody levels in the final years, as well as an ACPA repertoire maturation, before diagnosis of RA[3 4 6 7]. The present results are more in line with results of studies of RA patients shortly after the diagnosis, in which relatively stable levels of autoantibodies were observed on the group level[13 14].

The source population of the present study was seen at a late stage of disease development, which is also illustrated by the median of only two antibody measurements prior to development of arthritis. This could mean that the time window for detection of a change in autoantibody levels was too small, or that these levels had already reached the plateau typical of early RA[6].

The hazard ratio's for some of the clinical variables taken into account in the Cox proportional hazards survival model differs from earlier prediction models derived from the same cohort as well as from other cohorts, e.g. by the deletion of tender joint count and family history[10 15]. This may have been caused by the fact that patients developing arthritis shortly after inclusion (possibly for instance the ones with a high tender joint count or a positive family history for RA) in the study were not currently analysed, because they did not have at least two measurements of autoantibodies before arthritis occurred. However, considering the body of evidence that exists on the importance of these variables, we included them in the joint models regardless of statistical significance.

This study has some limitations, related to the chosen study population and interpretation of the study results. Thirteen patients, of whom three developed arthritis, were left out of the analyses because of one or more missing values in their baseline characteristics. However, since the percentage of excluded patients was only 5% we assume that this did not diminish the internal validity. The comparison between the cumulative/dynamic AUC of the Cox model with only baseline variables and the AUC based on the joint model should be interpreted with some caution, since they include the same prediction window, but refer to a slightly different time window of 0-3 years and 2-5 years respectively. Also, we quantified the AUC using the same data that was used to construct the model. Since we did not correct for over-optimism, and we had 14 covariates/parameters in our full prediction model with only 69 events, the AUC is likely to be inflated.

To our knowledge, investigation of the added value of repeated RF and ACPA levels for individual prediction of future arthritis in prospective studies has not been done before. Based on the current data, using time-updated autoantibody levels to differentiate between patients who will or will not develop arthritis seems to have little added predictive value in patients presenting with arthralgia. Possibly repeated autoantibody testing could be useful at an earlier stage of disease development, such as in asymptomatic individuals with seropositivity (e.g. first degree relatives of patients with RA).

In conclusion, using updated ACPA levels over time had only limited additional predictive value over baseline measurement of ACPA levels in this preselected cohort, while RF had no predictive value at all for development of arthritis in this cohort.

KEY MESSAGES:

1. As known, ACPA and RF autoantibody levels predict arthritis development in seropositive arthralgia patients
2. Also, higher time-updated ACPA levels were significantly associated with the development of arthritis
3. However, time-updated autoantibody levels have limited added predictive value over baseline measurements

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CHAPTER

Predicting onset of arthritis in individuals at risk for rheumatoid arthritis using dominant B cell receptor clones in peripheral blood

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ABSTRACT

Background: Of the individuals presenting with arthralgia and IgM rheumatoid factor and/or anti-CCP antibodies 28% will develop RA within 3 years. Recent studies showed that among these RA-risk individuals risk for arthritis was 83% in the subgroup that had 5 or more dominant B-cell receptor (BCR) clones. Here we analyse this association in more detail in a larger prospective cohort.

Methods: The BCR repertoire in peripheral blood was analysed using next-generation BCR sequencing in a prospective validation cohort study of 122 RA-risk individuals. 45 individuals were labelled BCR-positive since their peripheral blood at study baseline showed ≥ 5 dominant BCR-clones, defined as clones expanded beyond 0.5% of the total repertoire.

Results: Within 3 years none of the BCR-negative RA-risk individuals developed arthritis, while 32 (73%) of the BCR-positive individuals did (estimated RR: 114.1; 95%-CI: 7.2 - 1819; $p<0.0001$). Among the BCR-positives 91% of the individuals with 9 or more dominant clones (n=22; 18%) developed arthritis within 3 years, after a median follow-up of 16 months (BCR-high positive group), while among individuals with 5 to 8 dominant clones 55% developed arthritis (BCR-median positive group; log-rank test $p=0.006$).

Conclusion: Dominant BCR-clones in peripheral blood predict imminent onset of rheumatoid arthritis in seropositive arthralgia individuals with high accuracy. A higher number of dominant BCR-clones is significantly correlated with higher risk. We propose this BCR-test will support start of early intervention in BCR-high positive patients, supports retesting in BCR-medium positive patients, and may help rheumatologists to reassure BCR-negative individuals in an evidence-based way.

INTRODUCTION

A phase characterized by the presence of specific autoantibodies and arthralgias in the absence of clinically evident synovial inflammation often precedes the onset of rheumatoid arthritis (RA)[1-3]. However, only a subset, around 28%, of these *RA-risk* individuals will develop active disease in the short term[4]. This hinders the implementation of early interventions that may prevent onset of clinical disease. In response to this, various research groups have started developing prediction models. Some models include clinical, laboratory or imaging parameters and others combine different parameters[5-11]. For instance, in 2013 van de Stadt et al. developed a *risk rule model* (RRM) which includes nine domains, with both clinical and laboratory parameters[6]. Depending on their total score RA-risk individuals can be categorized in three risk groups: low, intermediate and high risk. The intermediate and high risk group had a hazard ratio of 4.52 and 14.86, respectively.

An example of a model that is based on one laboratory parameter is shown in a study from Tak et al[5]. Using RNA-sequencing they show that the presence of five or more dominant B-cell receptor (BCR) clones in peripheral blood accurately predicts imminent onset of arthritis in these RA-risk individuals: 83% of the individuals with a positive test result have arthritis within 3 years. This test significantly outperformed the RRM.

In this paper we replicate the BCR-clone test in a larger cohort of 122 individuals at risk for arthritis. We show that BCR-negative individuals have a risk comparable to background risk. In addition, we show that patients that harbor 9 or more dominant BCR clones show a very high risk for imminent onset of rheumatoid arthritis. We propose this test may be used to select individuals for early intervention studies in RA-risk individuals with high imminent risk, and may help rheumatologists to reassure BCR-negative individuals in an evidence-based way.

METHODS

Patients

Patients with a positive aCCP and/or IgM-RF status and (a history of) arthralgia, but not arthritis, and who were not part of the previous BCR study, were recruited at Reade, in Amsterdam, the Netherlands. The cohort study was approved by the local medical ethical committee of the MC Slotervaart and Reade, and all study subjects gave written informed consent prior to inclusion in the study. The study was performed according to the principles in the Declaration of Helsinki. Including and study visit procedures were similar to those described in the manuscript of van de Stadt et al[6]. In 122 of the included RA-risk individuals the BCR repertoire in peripheral blood was analyzed using next-generation BCR sequencing. Patient characteristics of these RA-risk individuals are shown in table 1. All patients were scored using the risk rule model (RRM) developed by van de Stadt et al. The RRM consists of nine clinical parameters categorising individuals into a low, intermediate or high risk group. The separate parameters are presented in table 1.

Table 1. Patient characteristics (n=122) FDR, first degree relative; IgM-RF, IgM rheumatoid factor; aCCP, anti-cyclic citrullinated peptide antibodies; ns, not significant; RA, rheumatoid arthritis; RRM, risk rule model; VAS, visual analogue scale.

Variable	Arthritis yes (n=45)	Arthritis no (n=77)	p
Age, years, mean (SD)	49.2 (10.4)	49.1 (12.2)	ns
Male, nr (%)	11 (24%)	15 (19%)	ns
FDR with RA, nr (%)	13 (29%)	9 (12%)	0.0169
No alcohol, nr (%)	19 (42%)	23 (30%)	ns
Duration of symptoms <12 months, nr (%)	14 (31%)	19 (25%)	ns
Intermittent symptoms present, nr (%)	18 (40%)	17 (22%)	0.0349
Arthralgia in upper and lower extremities, nr (%)	22 (49%)	33 (43%)	ns
VAS pain ≥50, nr (%)	15 (33%)	23 (30%)	ns
Morning stiffness ≥1 h, nr (%)	10 (22%)	15 (19%)	ns
Swollen joint(s) reported, nr (%)	18 (40%)	14 (18%)	0.0223
Antibody status, nr (%)			
IgM-RF positive, aCCP negative	3 (7%)	43 (56%)	<0.0001
aCCP low positive, IgM-RF negative	7 (16%)	13 (17%)	ns
aCCP high positive, IgM-RF negative	14 (31%)	11 (14%)	0.0263
aCCP and IgM-RF positive	21 (47%)	10 (13%)	<0.0001
Total score RRM, mean (SD)	6.3 (2.1)	3.6 (2.0)	<0.0001
Low risk on RRM (0-4 points), nr (%)	7 (16%)	57 (74%)	<0.0001
Intermediate risk on RRM (5-6 points), nr (%)	17 (38%)	14 (18%)	0.0335
High risk on RRM (7-13 points), nr (%)	21 (47%)	6 (8%)	<0.0001

Linear amplification and next-generation sequencing

The protocol used for linear amplification, next-generation sequencing and bioinformatics has been described before[5]. As was done in the previous study BCR-clones that were expanded beyond 0.5% of the total repertoire were labeled dominant. Based on the initial study, individuals in this validation cohort were considered positive if peripheral blood at study baseline showed ≥ 5 dominant BCR-clones.

Statistics

Data evaluation and statistical analysis were performed with the R software environment (version 3.1.0) and Graphpad Prism software (version 6.0). Differences between groups and predictive tools were analyzed using the unpaired t test, delta Akaike information criterion (ΔAIC), Spearman rank correlation, log-rank test, likelihood-ratio test, R^2, Cox proportional hazard ratio and relative risk (RR). In addition, we introduced an "estimated RR", since none of the RA-risk individuals who tested BCR-negative developed arthritis within 3 years.

This estimated RR was only used for this particular analysis and was calculated by adding 0.5 points to all groups of the formula (a, b, c and d)[12]:

$$RR = \frac{a/(a+b)}{c/(c+d)}$$

The *p* values <0.05 (two-sided) were considered statistically significant.

RESULTS

Validating the BCR-test

Dominant BCR-clones predict onset of arthritis in RA-risk individuals[5]. In the current cohort we included a new cohort of 122 RA-risk individuals of whom 32 developed arthritis within 3 years and 45 after the total follow-up of 8,5 years. This provides us with enough events per variable (EPV) to validate the predictive properties of this *BCR-test*. We observed that the number of dominant BCR-clones were increased in RA-risk individuals who developed arthritis, compared to RA-risk individuals who did not develop arthritis (mean 10.5 (±5.2), vs. 2.0 (±2.5), p<0.0001, figure 1A). Also the impact of all dominant BCR-clones combined on the total BCR repertoire was increased in RA-risk individuals who developed arthritis, compared to RA-risk individuals who did not develop arthritis (mean 17.0 (±14.3) vs. 4.1 (±12.1) respectively, p<0.0001, data not shown). When creating a receiver operating characteristic (ROC) curve for this test on the present cohort we could replicate the most optimal cut-off of ≥ 5 dominant BCR-clones in the peripheral blood (figure 1B). This cut-off divided the cohort in two groups, hereafter called BCR-positive individuals (n=45) and BCR-negative individuals (n=77). Using this cut-off to predict onset of arthritis within 36 months corresponded with a sensitivity of 100%, specificity of 87%, positive predictive value (PPV) of 73%, negative predictive value (NPV) of 100% and an estimated relative risk (RR) of 114.1 (figure 1D, figure 1C shows the Kaplan-Meier curve for a follow-up time of 40 months, log-rank test p<0.0001). Interestingly, none of the BCR-negative individuals, 63% of the cohort, developed arthritis within 36 months. When looking at the total follow-up of 104 months only 13% of the BCR-negative individuals developed arthritis comparing to 76% of the BCR-positive individuals, resulting in a RR of 5.8 (95%-CI 3.2-10.6, p<0.0001).

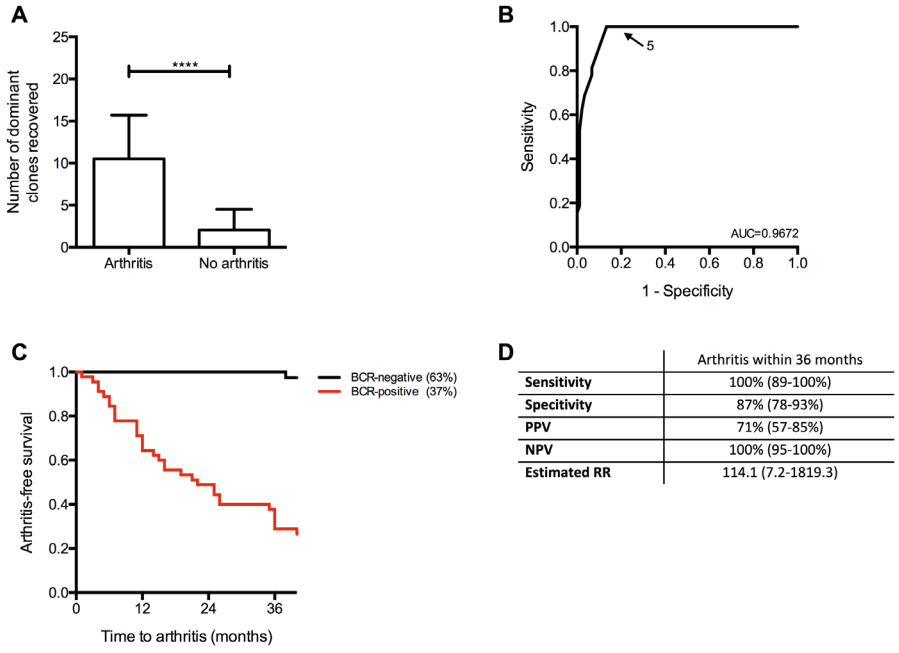


Figure 1. Validation of predictive properties of highly expanded BCR-clones in RA-risk individuals (n=122) who either did or did not develop arthritis within 3 years Bar charts of (A) the absolute number of dominant BCR-clones (clonal size ≥ 0.5% of the total repertoire), in RA-risk individuals that developed arthritis (n=32) versus RA-risk individuals that did not develop arthritis (n=90) within 36 months. Bars show mean and SD, **** p<0.0001 using an unpaired t test. (B) Receiver Operating Characteristic (ROC) curves for the number of HECs. AUC= area under the curve. (C) Arthritis-free survival curve for BCR-positive and BCR-negative individuals. (D) Table describing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and estimated relative risk (RR) including 95% confidence intervals for the BCR-test, based on whether individuals developed arthritis within 36 months.

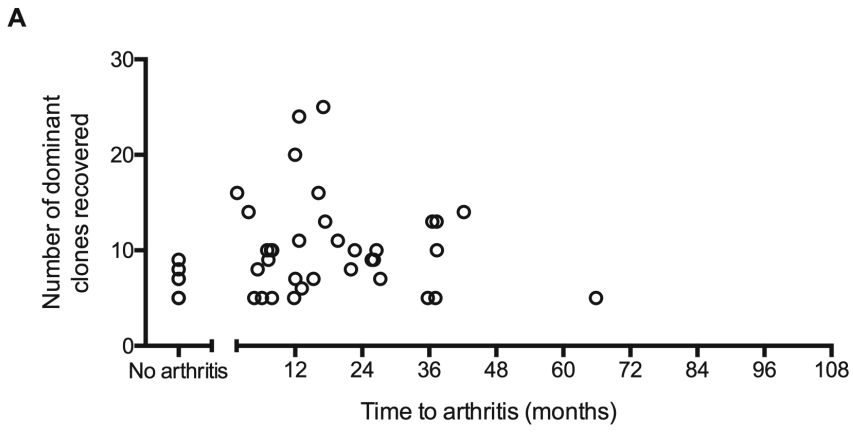
Does a higher number of highly expanded BCR-clones predict onset of arthritis even more accurately?

It has been observed that the number of antigen specific clones increases before onset of arthritis. We hypothesized that a higher number of dominant BCR-clones therefore would predict onset of arthritis even more accurately. This could be confirmed, even when this analysis was restricted to the BCR-positive group only (Spearman r -0.31, 95%-CI 0.003-0.56, p=0.0418, figure 2A).

When performing a logistic regression, the model with the number of HECs was significantly better fitted for the prediction of the development of arthritis in comparison

to being either BCR-positive or BCR-negative or the impact of all HECs (ΔAIC= 5.8 and 102.05 respectively). Because the frequency of individuals decreased with the increase of number of HECs, we decided to divide BCR-positive individuals into two equal groups for further analyses, namely individuals with 5-8 HECs (e.g. BCR-medium positive individuals, n=22) or with 9 or more HECs (e.g. BCR-high positive individuals, n=22), each 18% of the at risk individuals. At 36 months 91% of the individuals in the BCR-high positive group had developed arthritis. In the BCR-medium positive group this was 55%. As said before in the BCR-negative group there were no individuals who developed arthritis within 3 years. Using a logistic regression we found that splitting the group into these three groups was a better fitted predictive model than dividing the cohort in the initial two categories (ΔAIC= 9.13). The model was similar to using the absolute number of HECs (ΔAIC= 3.33). The test where the results were split into three BCR-groups had an Cox proportional hazard ratio of 5.9 (95%-CI 3.8-9.1).

In comparison with a predictive tool that is based on clinical parameters, namely the risk rule model (RRM), the BCR-test performed significantly better in predicting development of arthritis. In the current cohort 64 individuals could be categorised into the low risk, 31 in the intermediated risk and 27 in the high risk group. Alone the RRM had a hazard ratio of 3.4 (95%-CI 2.3-5.1). Using a logistic regression the BCR-test was significantly better in predicting development of arthritis in comparison with the RRM (ΔAIC= 15.67). When looking into more detail into the group of individuals that scored high on the RRM, we could still divide this group into the three categories of the BCR-test. The patient characteristics did not differ significantly, except for arthritis development within 3 years (table 2, one-way ANOVA p<0.0001).



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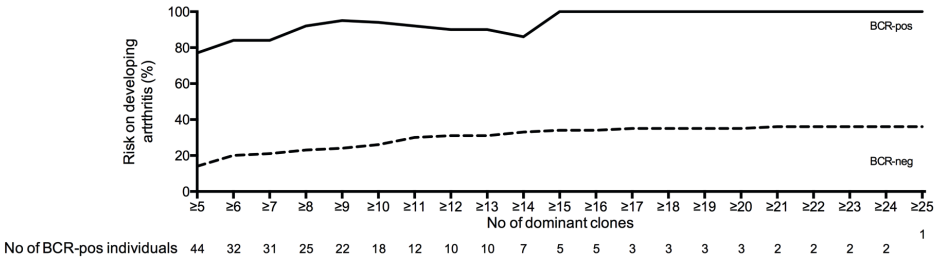

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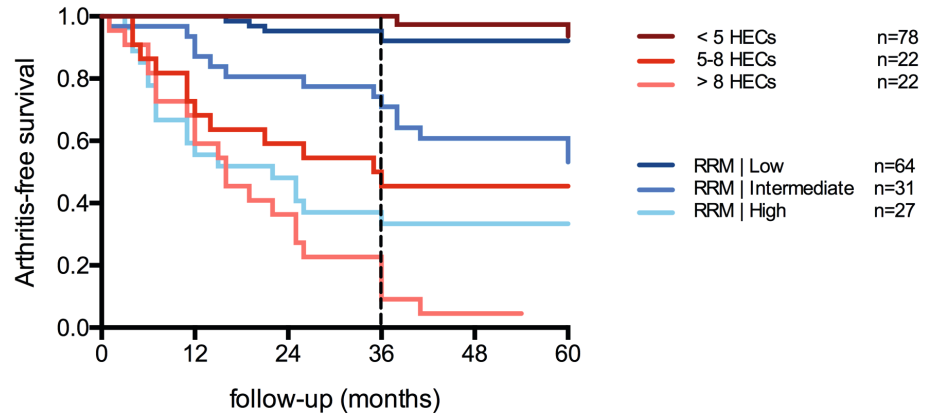

Figure 2. Correlation between number of highly expanded clones and time to arthritis (A) Scatter plot of the absolute number of dominant BCR-clones (clonal size ≥ 0.5% of the total repertoire) and time to arthritis per BCR-positive RA-risk individuals (n=45) during the complete follow-up. (*) FYI Line plot of delta BCR-positive and BCR-negative individuals with different cut-offs. (**) FYI Arthritis-free survival curve when the RA-risk individuals are divided into 3 subcategories of the BCR-test (red lines), or when the individuals are divided into the 3 subcategories of the risk rule model (blue lines)

Table 2. Patient characteristics from individuals who had a high risk score on the RRM (n=27) FDR, first degree relative; IgM-RF, IgM rheumatoid factor; aCCP, anti-cyclic citrullinated peptide antibodies; ns, not significant; RA, rheumatoid arthritis; RRM, risk rule model; VAS, visual analogue scale.

Variable	BCR-high positive (n=12)	BCR-medium positive (n=7)	BCR-negative (n=8)	p
FDR with RA, nr (%)	6 (50%)	1 (14%)	2 (25%)	ns
No alcohol, nr (%)	7 (58%)	4 (57%)	2 (25%)	ns
Duration of symptoms <12 months, nr (%)	5 (42%)	3 (43%)	2 (25%)	ns
Intermittent symptoms present, nr (%)	7 (58%)	3 (43%)	5 (63%)	ns
Arthralgia in upper and lower extremities, nr (%)	7 (58%)	5 (71%)	6 (75%)	ns
VAS pain ≥50, nr (%)	5 (42%)	4 (57%)	3 (38%)	ns
Morning stiffness ≥1 h, nr (%)	5 (42%)	2 (29%)	2 (25%)	ns
Swollen joint(s) reported, nr (%)	7 (58%)	5 (71%)	6 (75%)	ns
Antibody status, nr (%)				
IgM-RF positive, aCCP negative	0 (0%)	0 (0%)	0 (0%)	ns
aCCP low positive, IgM-RF negative	0 (0%)	0 (0%)	0 (0%)	ns
aCCP high positive, IgM-RF negative	3 (25%)	3 (43%)	3 (38%)	ns
aCCP and IgM-RF positive	9 (75%)	4 (57%)	5 (63%)	ns
Developed arthritis within 3 years, nr (%)	12 (100%)	6 (86%)	0 (0%)	<0.0001

DISCUSSION

Using next-generation whole-repertoire B-cell receptor analysis in a RA-risk cohort we confirmed that the presence of dominant BCR-clones predicts onset of arthritis with high accuracy. This cohort was used as a validation for results earlier described by Tak et al[5]. Moreover, the BCR-test could reassure 65% of the RA-risk cohort as none of the BCR-negative individuals developed arthritis within 3 years. Which means that the imminent risk for arthritis is similar to the population background risk[13].

Furthermore, we showed that a high number of highly expanded BCR-clones could predict imminent onset of arthritis in 18% of the individuals with very high accuracy (3-year positive predictive value 91%). This supports the idea that the number of BCR-clones increases before onset of arthritis in the peripheral blood. Earlier studies showed that B-cells migrate to the inflamed synovium once arthritis becomes apparent[5 14]. It is thought that this could be due to a “second hit”, such as a trauma or viral infection, that causes the arthritis and subsequent migration of B-cells to the synovium[2 15].

The strong predictive value of a high number of clones indicates that a BCR-high status might be an indication for preventive treatment. Recent research already showed that

treatment during the early, therapeutic window of opportunity leads to more effective and often drug-free remission[16]. Furthermore, such a strategy might help to prevent the substantial increase in sick leave starting long before diagnosis of RA in a substantial proportion of the RA-risk individuals[17]. Both effects might help to reduce the huge societal costs of this disease[18]. Clinical trials also have taken up this idea and started treating RA-risk individuals with rituximab, abatacept and simvastatin. Preliminary data from the RA-risk cohort treated with rituximab showed that development of arthritis could be delayed with 12 months[19]. For future perspectives it would be very interesting to see whether this effect would be even larger when only the BCR-high positive individuals were treated during this window of opportunity. If so, it will only be a matter of time before treating RA-risk individuals will be the Golden Rule in out-patient clinics, making prediction models more important than ever.

The BCR-test used is a very specific test which uses next-generation sequencing. The latter is widely available in most academic hospitals or specialized sequencing laboratories. Other out-patient clinics, for instance (smaller) peripheral hospitals or specialized rheumatology centres, would therefore need to transfer blood to such facilities for analysis. This could cause delay in the risk assessment. However, looking at our own sequencing capacity, results can be available within 2-3 weeks. Compared to the 36 months in which arthritis can become apparent, it is safe to say that this delay is limited.

In conclusion, we replicated that dominant BCR-clones in peripheral blood predict onset of clinical symptoms of RA in RA-risk individuals with high accuracy. Our data show that individuals who are BCR-negative can be reassured, since they have a risk for arthritis similar to the background population risk. In contrast, RA-risk individuals in whom analysis of peripheral blood shows 9 or more dominant BCR clones are at imminent risk for arthritis (91% within 3 years). In these RA-risk individuals preventive treatment might be indicated, pending further studies showing efficacy of the selected drugs in this patient group in this disease phase.

ACKNOWLEDGMENTS AND AFFILIATIONS

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CHAPTER 10

The value of joint ultrasonography in predicting arthritis in seropositive arthralgia patients: a prospective cohort study

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ABSTRACT

Background: The value of joint ultrasonography (US) in the prediction of clinical arthritis in individuals at risk of developing rheumatoid arthritis (RA) is still a point of debate, due to varying scanning protocols and different populations. We investigated whether US abnormalities assessed with a standard joint protocol can predict development of arthritis in seropositive arthralgia patients.

Methods: Anti-citrullinated protein antibodies and/or rheumatoid factor positive patients with arthralgia, but without clinical arthritis were included. US was performed at baseline in 16 joints: bilateral metacarpophalangeal 2-3, proximal interphalangeal 2-3, wrist and metatarsophalangeal (MTP) joints 2-3 and 5. Images were scored semi quantitatively for synovial thickening and Power Doppler (PD). Associations between US abnormalities and arthritis development at the joint and patient level were evaluated. Also, we investigated the added value of US over clinical parameters.

Results: Out of 163 patients who underwent US examination, 51 (31%) developed clinical arthritis after a median follow-up time of 12 (interquartile range 5-24) months, of which 44 (86%) satisfied the 2010 ACR/EULAR classification criteria for RA. US revealed synovial thickening and PD in at least one joint in 49 (30%) and 7 (4%) of the patients, respectively. Synovial thickening was associated with both development and timing of clinical arthritis in any joint (patient level) when MTP joints were excluded from the US assessment (odds ratio 6.6, confidence interval (CI) 1.9-22), and hazard ratio 3.4, CI 1.6-6.8, respectively, with a mean time to arthritis of 23 versus 45 months when synovial thickening was present versus not present). There was no association between US and arthritis development at the joint level. Predictive capacity was highest in the groups with an intermediate and high risk of developing arthritis based on a prediction rule with clinical parameters.

Conclusions: Synovial thickening on US predicted clinical arthritis development at the patient level in seropositive arthralgia patients when MTPs were excluded from the US assessment. PD was infrequently seen in these at-risk individuals and did not predict. In patients at intermediate risk for RA, US may help to identify those at higher risk of developing arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease that is characterized by synovial inflammation and swelling. In the at risk phase before clinical RA development, the presence of autoantibodies such as anti-citrullinated protein antibodies (ACPA) and/or IgM-rheumatoid factor (RF) with or without arthralgia symptoms are predictive for RA development[1-4]. Early treatment of RA improves the outcome[5], and this principle may also apply to the preclinical phase of RA. Detecting arthralgia patients at high risk for RA offers the opportunity to develop treatment strategies for prevention of RA in these patients. Current prediction rules for arthritis development based on clinical parameters (including autoantibodies) are suitable for this[4 6-8], but their predictive value seems too low to ensure that all patients would be treated validly with medication with potentially serious side effects. The predictive capacity might be substantially improved by adding imaging[9].

Ultrasonography (US) is widely available at relatively low cost and has no radiation exposure. There is evidence that US increases diagnostic certainty when compared to clinical examination alone for diagnosing RA in early undifferentiated arthritis[10-15]. US was also described to add value to clinical examination in individuals at risk of developing RA[9 11 16-20], which may be particularly the case for power Doppler (PD) abnormalities[9 11 19] and mainly in autoantibody negative persons[17 18]. However, discrepancies related to the definition of US synovial thickness[21], the selection of joints included in the US protocol[21] and the use of different scoring systems[22-25] hamper general clinical implementation of US to help diagnose and predict RA[26].

In a previous study on the value of US in the prediction of arthritis in seropositive arthralgia patients, we only scanned painful and adjacent/contralateral joints (which differed between patients) and showed that arthritis could be predicted at the joint but not at the patient level[16]. The present follow-up study included a new cohort of seropositive arthralgia patients, in which we investigated the value of an US protocol including a standardized set of joints (regardless of local clinical symptoms) to predict clinical arthritis development. We also evaluated whether US abnormalities add predictive value over clinical parameters.

METHODS

Study population

Seropositive arthralgia patients (ACPA and/or RF), but without clinical arthritis, were recruited at Reade (Amsterdam) between March 2009 and December 2015. Patients with past arthritis or arthritis at baseline (defined as 1 or more swollen joints as reported by two independent investigators), age <18 years and >70 years, previous treatment with a disease modifying anti-rheumatic drug or recent glucocorticoid treatment, systemic autoimmune disease, systemic infections, lymphoproliferative disorders or recent radiotherapy were excluded from the cohort[2 16]. Medical history, tender joint count of 53 joints (TJC53), details of joint symptoms and ACPA/RF status were recorded at baseline[2], together with clinical criteria included in a previously described prediction rule for the development of arthritis

in seropositive arthralgia patients: presence of a first degree relative with RA, alcohol consumption, symptom onset <12 months, presence of intermittent symptoms, presence of symptoms in upper and lower extremities, presence of joint swelling (anamnestic), visual analogue scale assessing pain (≥50 mm) and morning stiffness of at least 1 hour[4]. These parameters (combined with the autoantibody status) were used to calculate a risk rule score ranging from 1 to 13, divided in three risk groups (low 0-4, intermediate 5-6, high 7-13). During yearly follow up, up to 5 years, clinical arthritis development in any of 44 joints was assessed by a trained physician and an extra visit could be scheduled when arthritis development was suspected. If clinical arthritis was present in at least one joint, confirmation was done by a senior rheumatologist without knowledge of serostatus (DvS). The study was approved by the Slotervaart ziekenhuis and Reade ethics committee. Signed informed consent was obtained from all patients prior to inclusion.

Ultrasonography

Joints were scanned according to a predefined standard US protocol of those 16 joints in which clinical swelling had developed most often in our previous US pre-RA cohort: bilateral wrists, metacarpophalangeal (MCP) 2-3, proximal interphalangeal (PIP) 2-3 and metatarsophalangeal (MTP) 2-3 and 5[16]. All scans were performed with 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 greyscale and 8.9 MHz for PD) and VF 13-5 for larger joints (operating at 11.43 MHz for greyscale and 7.3 MHz for PD), according to the manufacturer's criteria[16]. Joints were scanned in the dorsal longitudinal plane from the most lateral to the most medial site and in the transverse plane from the proximal to distal site of the joint. Finger joints were also scanned in the palmar longitudinal plane. The wrist included scans of the radiocarpal and intercarpal joints and ulnocarpal joint including the ulnar styloid process. Effusion and synovial hypertrophy were scored in a combined measure (synovial thickening) as both phenomena often appear concurrently[27]. Synovial thickening and PD were scored using the four-grade semi quantitative scale (0-3) by Szkudlarek[16 22]. Synovial thickening grade ≥2 and PD grade ≥1 were regarded as abnormal. When multiple images were made of one joint, the highest score was used to obtain a single score per joint. US examinations were all performed by a single radiologist (MMR) experienced in musculoskeletal US, who was blinded for the clinical data.

Statistics

Continuous data with a normal distribution were summarized with mean and standard deviation (SD). Non-normally distributed data were summarized with median and interquartile range (IQR). The risk of arthritis development at the patient level was estimated by Chi-square or Fisher's exact test, and corresponding positive and negative predictive values (PPV, NPV) were calculated. Results were expressed as odds ratios (OR) with 95% confidence interval (CI). Timing of arthritis development was assessed with Kaplan Meier survival analysis with log-rank test and Cox regression analysis, expressed as mean time to arthritis (we reported mean survival time instead of the mostly preferred

median survival time, because in order to calculate the median 50% needs to develop arthritis and this was not the case in any of our groups) and hazard ratios (HR) with 95% CI. We also performed multivariate regression analysis to look at the additional value of US over clinical parameters in the patients with low, intermediate or high risk of developing RA[4]. Subgroup analyses were performed for ACPA positive versus ACPA negative patients. All analyses at the patient level were performed with and without inclusion of the MTP joints, since a previous study indicated that the frequency of synovial thickening in the MTP joints in healthy controls was too high to discriminate between those who will develop arthritis and those who do not[19]. The risk of arthritis development at the joint level (using all joints) was analyzed using generalized estimating equations (GEE) with an exchangeable correlation matrix, allowing correction for within-patient correlation[28]. Statistical analysis was performed with SPSS version 22 statistics software (SPSS Inc., Chicago, IL, USA).

RESULTS

In total, 287 consecutive seropositive arthralgia patients were screened through our prospective cohort in the inclusion period. Fourteen were excluded due to clinical arthritis at baseline, 99 patients did not receive US examination due to logistical problems or no consent to make an US and 11 patients were lost-to-follow up after their baseline measurement. The remaining 163 patients were analyzed in the current study (74% female, mean ± SD) age 51 ± 11 years). Their baseline characteristics are shown in **Table 1**. Baseline characteristics were similar for those who were included compared to those who were not (data not shown). Fifty-one (31%) patients developed clinical arthritis after a median follow-up of 12 (IQR 5-24) months. Forty-four (86%) patients developing arthritis satisfied the 2010 ACR/EULAR classification criteria for RA. The 112 patients who did not develop arthritis had a median follow-up time of 28 (IQR 19-49) months. US was performed within a median of 3 weeks (IQR 2-6 weeks) after the first visit.

Table 1. Baseline characteristics

Baseline characteristics	n = 163
Age in years, mean ± SD	51 ± 11
Female sex, n (%)	121 (74%)
Arthralgia duration in months, median (IQR)	13 (6-36)
Number of reported painful joints, median (IQR)	8 (4-19)
Tender joint count (53 joints), median (IQR)	1 (0-5)
VAS pain in mm (0-100), mean ± SD	35 ± 25
Antibody status	
ACPA negative, RF positive, n (%)	72 (44%)
ACPA positive, RF negative, n (%)	44 (27%)
ACPA positive, RF positive, n (%)	47 (29%)

ACPA, anti-citrullinated protein antibodies; IQR, interquartile range; RF, rheumatoid factor; SD, standard deviation; VAS, visual analog scale.

US and clinical arthritis development at the patient level
At baseline, 49 (30%) patients had US synovial thickening and 7 (4%) patients PD in at least one joint (**Table 2**). Of these, 5 (3%) patients had both synovial thickening and PD in at least one joint and 3 (2%) had both synovial thickening and PD in the same joint (with 1 patient having 4 joints with both synovial thickening and PD). When excluding the MTP joints, 14 (9%) patients had synovial thickening in at least one joint and 7 (4%) PD.

Of the patients with US abnormalities in at least one joint, the median number of affected joints with synovial thickening was 2 (min-max 1-6) in the patients developing arthritis and also 2 (min-max 1-4) in the patients who did not develop arthritis. For PD these numbers were 1 (1-5) and 3 (1-3), respectively.

A greater proportion of patients with US synovial thickening at baseline in at least one joint developed arthritis although this did not reach statistical significance (**Table 2**). This trend appeared to be more pronounced and reached significance when MTP joints were excluded (OR 6.6, CI 1.9-22.2, p<0.01). Corresponding PPV and NPV were 71% and 72%. There was no statistically significant association between the presence of PD in one or more joints and clinical arthritis development (OR 0.9, CI 0.1-4.7, p=1.0, PPV 29%, NPV 69%). All patients with PD in the MTP joints also had PD in at least one other joint, therefore the association did not change when MTP joints were excluded.

Sensitivity analysis with RA development according to the ACR/EULAR 2010 classification criteria as outcome showed a slight increase in odds ratios and predictive values as compared to clinical arthritis as outcome (OR 8.5, CI 2.4-28.7, p=<0.01, PPV 71%, NPV 77%; **Table 2**).

Clinical arthritis development occurred earlier in patients who had US synovial thickening in at least one joint than in those without US synovial thickening, but this was only the case when the MTP joints were excluded from the US assessment (mean time to arthritis 23 versus 45 months, p<0.01; **Figure 1**). The corresponding HR was 3.4 (CI 1.6-6.7, p<0.01). Patients with PD (both with and without MTP joints) did not develop arthritis earlier than patients without PD (mean time to arthritis 44 versus 43 months, p=0.7; **Figure 1**) and the corresponding HR of 0.8 (CI 0.1-3.2, p=0.7) was not statistically significant. In Figure 1B due to low numbers of PD positive patients, the lines were crossed. Since this could be caused by effect modification, we investigated whether there was a significant difference between the effects of patients before a cut-off value of 30 months and after, which was not present.

We could not demonstrate clinically relevant differences in US abnormalities for prediction of arthritis at the patient level between ACPA positive and ACPA negative patients, except for synovial thickening without MTP joints in the ACPA positive patients (19% developed arthritis versus 2% who did not; note the small numbers and thus wide CI's, **supplementary Table 1**).

Table 2. Association of ultrasound abnormalities with clinical arthritis/rheumatoid arthritis development at the patient level

Ultrasound abnormalities	Arthritis yes	Arthritis no	OR (95% CI)	p-value	PPV	NPV
Outcome: *clinical arthritis*	n=51	n=112				
Synovial thickening* (16 joints)	19 (37%)	30 (27%)	1.6 (0.8-3.3)	0.18†	39%	72%
Synovial thickening (10 joints, no MTP)	10 (20%)	4 (4%)	6.6 (1.9-22.2)	<0.01‡	71%	72%
Power Doppler* (16 joints)**	2 (4%)	5 (5%)	0.9 (0.1-4.7)	1.0.‡	29%	69%
Outcome: *2010 RA criteria*	n=44	n=119				
Synovial thickening (16 joints)	17 (39%)	32 (27%)	1.7 (0.8-3.6)	0.15†	35%	76%
Synovial thickening (10 joints, no MTP)	10 (23%)	4 (3%)	8.5 (2.4-28.7)	<0.01‡	71%	77%
Power Doppler (16 joints)**	2 (5%)	5 (4%)	1.1 (0.2-5.9)	1.0‡	29%	73%

† Chi-square test, ‡ Fisher's exact test.

* Results are presented for synovial thickening and Power Doppler in at least one joint

** Same results when excluding MTP joints

CI, confidence interval; MTP, metatarsophalangeal; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; RA, rheumatoid arthritis.

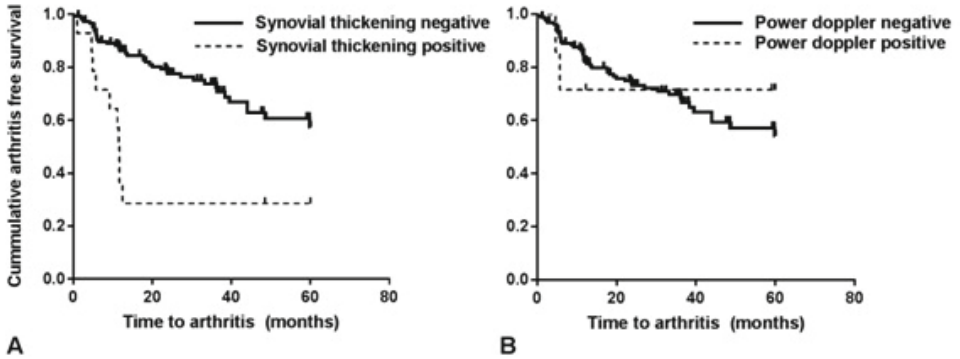

Figure 1. Kaplan Meier curves for synovial thickening (A) and Power Doppler (B) and time to arthritis development (months). The MTP joints were excluded

US and clinical arthritis development at the joint level

At baseline, US was performed on 2608 joints (16 joints per patient, no missing data). US revealed synovial thickening in 112/2608 (4%) of the joints, mostly in MTP joints (91/112 (81%)). PD was seen in 14/2608 (0.5%) of the joints (of which 3/14 (21%) in MTP joints). In 158/2608 (6%) of the scanned joints at baseline, clinical arthritis developed during follow-up (32% of this in MTP joints). At that moment, the median number of joints with arthritis per patient was 3 (range 0-13, note that in 5 patients arthritis development was found outside the standard set of 16 joints). Of the 158 joints with arthritis, only 8 (5.1%) had synovial thickening on US in the same joint at baseline (for PD this was 1 joint (0.6%)). No statistically significant association was found with either the presence of synovial thickening in a joint and arthritis development in the same joint (OR 1.0, CI 0.3-2.9, p=1.0) or the presence of PD (OR 1.0, CI 0.2-4.8, p=1.0).

Added predictive value for clinical arthritis of US over clinical parameters

Patients were divided into three groups of low, intermediate and high risk of developing arthritis according to the clinical prediction rule score described in the method section. Multivariate regression analysis including the clinical prediction rule risk groups and synovial thickening in all joints excluding the MTP joints showed an OR of 6.1 (CI 1.6-23.2, p<0.01) for synovial thickening and 3.5 (CI 2.2-5.5, p<0.01) for the prediction rule groups. The number of patients in each group was too low to perform proper subgroup analysis, however, the relatively high OR of 6.1 of synovial thickening seems to be caused by both the patients from the intermediate risk group (in which four patients with synovial thickening developed arthritis and only one did not), and the high risk group (in which all six patients with synovial thickness also developed arthritis, see **Table 3**). For PD the OR in multivariate regression was 1.7 (0.3-10.2, p=0.55), with an OR of 3.6 (2.3-5.6, p<0.01) for the clinical score.

Table 3. Added value of ultrasound over clinical parameters according to a clinical prediction rule

Ultrasound abnormalities *Clinical prediction rule risk groups**	Arthritis yes	Arthritis no	OR (95% CI)†	p-value
Synovial thickening** (16 joints)	n=51	n=112	1.5 (0.7-3.4)	0.3
*Clinical prediction rule**			3.5 (2.2-5.4)	<0.01
Low risk	2/8 (25%)	19/67 (28%)		
Intermediate risk	5/13 (38%)	5/25 (20%)		
High risk	12/30 (40%)	6/20 (30%)		
Synovial thickening (10 joints, no MTP)			6.1 (CI 1.6-23.2)	<0.01
Clinical prediction rule			3.5 (CI 2.2-5.5)	<0.01
Low risk	0/8 (0%)	3/67 (4%)		
Intermediate risk	4/13 (31%)	1/25 (4%)		
High risk	6/30 (20%)	0/20 (0%)		
Power Doppler** (16 joints)***			1.7 (0.3-10.2)	0.5
Clinical prediction rule			3.6 (2.3-5.6)	<0.01
Low risk	0/8 (0%)	4/67 (6%)		
Intermediate risk	2/13 (15%)	1/25 (4%)		
High risk	0/30 (0%)	0/20 (0%)		

† Logistic regression analysis (note that the prediction rule risk groups were combined)

* Risk groups based on the clinical prediction rule described in reference number 4

** Results are presented for synovial thickening and Power Doppler in at least one joint (present, %)

*** Same results when excluding MTP joints

ACPA, anti-citrullinated protein antibody; CI, confidence interval; MTP, metatarsophalangeal; OR, odds ratio; RF, rheumatoid factor

DISCUSSION

Here we investigated whether abnormalities found with a standardized US protocol are useful in the prediction of arthritis development in seropositive arthralgia patients, and whether these US abnormalities add predictive value over clinically available parameters. Synovial thickening on US (wrist and hand joints, excluding MTPs) was associated with both arthritis development and its timing at the patient level, but not at the joint level. Also, US synovial thickening in wrist and hand joints adds predictive value in patients with an intermediate to high risk of developing arthritis based on a clinical prediction rule. PD abnormalities on US were not associated with arthritis development.

The results should be interpreted in the light of low numbers of patients with US abnormalities, especially for PD. Thirty-one percent of the patients had abnormalities on US in at least one joint, a number that decreased to 10% when not analyzing the MTP joints. In total, only 4% presented with PD. Therefore, even in our population with a relatively high risk of developing arthritis (around 30%), a large number needs to be screened to find only a small proportion of patients with US abnormalities that progress to arthritis. This is

undesirable for clinical implementation of US.
The usefulness of US as a predictor of arthritis or RA development has been described by several authors with varying results[9 11 16 18 19]. Our previous report of evaluating only painful joints concluded that presence of US abnormalities (both synovial thickness and PD) was associated with arthritis development at the joint level, but not at the patient level, which is opposite to our present conclusion[16]. A group from Leeds performed a study which included 136 ACPA positive patients with musculoskeletal symptoms and showed that synovial thickening in 2 or more joints was related to a 2.3 times higher chance of developing arthritis at the patient level, which increased to 3.7 for PD in at least 2 joints[19]. The hazard ratios were even higher when analyzing on joint level (HR 9.4 for synovial thickening score ≥2 in a joint and HR 31 for PD≥2). In another study, the same group found that PD signal added predictive value to clinical parameters in the prediction of arthritis [9] The higher scores (namely for PD) compared to our study may have been caused by the selection of patients with a higher a priori risk, as they were all ACPA positive arthralgia patients. A study from Switzerland focused on very early arthritis and evaluated 49 patients with inflammatory hand symptoms of recent onset (≤12 weeks) with or without clinical arthritis. Since all ACPA and/or RF positive patients eventually developed arthritis, the value of US was only determined in the seronegative patients[18]. In this subgroup the post-test probability in patients with 1-3 clinical parameters could be raised from 2-30% to 50-94% when using US synovial thickness or PD. Finally, synovial thickness (PD not analyzed) was also researched in another seronegative patient population of 80, in which the OR of arthritis and/or RA development was 7.5 (clinical parameters not taken into account)[18].

Three main reasons may have caused the different results presented above. Firstly, different US scanning protocols were used. Our study in combination with our previous study indicates that applying a US protocol with a standardized set of joints results in a better prediction at the patient level and that scanning only painful joints results in better prediction at the joint level. Although it appears attractive to scan more joints, both symptomatic and asymptomatic, this will make US more time-consuming and thus unfit for a clinical setting[29]. Also, it is still hard to define which set of asymptomatic joints should be used, but this study and another have shown that it may be useful to exclude the MTP joints[19]. This would be convenient since it lowers the time to make an ultrasound. The second reason for different results are technical differences between US machines, which mainly seems to be important for detection of PD signal[30]. Differences between groups may be overcome in the future as availability of good quality US machines increases. The final reason is the fact that the use of US in prediction depends highly on the a priori chance of developing arthritis in the population investigated. In patients with an already high risk, for instance those being ACPA positive (for example 42% developed arthritis in the Leeds cohort[19], 46% in the present study) or those with a high probability based on clinical prediction rules, having US abnormalities was almost always associated with arthritis development (in the present study six, with a 100% chance of developing arthritis)[9 17 19]. However, US might be of even more value in those subpopulations of at risk patients in which there is more diagnostic uncertainty, such as in seronegative arthralgia patients[17 18] and patients scoring intermediate on the clinical prediction rule. We did not include

seronegative patients, but we did show that of 5 patients with an intermediate risk of developing arthritis 80% had US abnormalities.

Some additional comments can be made. Firstly, it may be interesting to look not only at US abnormalities, but also to the absence of these in relation to a lower chance of developing arthritis. van der Ven et al[31], described a NPV of 89% of grey scale and/or PD abnormalities in 196 inflammatory arthralgia patients. In the present study, somewhat lower NPV's were found for synovial thickness (72%) and PD (77%) separately, although they were measured in a cohort with a low prevalence of US abnormalities. Secondly, it may be worthwhile to include tenosynovitis as an independent variable besides synovitis when looking at US abnormalities in the at-risk phase of RA[32]. Thirdly, it was speculated that US is of greater value when applying the 2010 criteria for RA, because this criteria set is designed to identify RA at an early stage[11]. This was confirmed by our study as both OR and NPV increased when the 2010 criteria for RA were used as outcome measure. Lastly, a limitation of this study may be that US examinations were all performed by a single radiologist, although this radiologist had a high interobserver agreement (88 to 92%) in our previous study[16].

In conclusion, synovial thickening on US using a standard US protocol with exclusion of MTPs predicted arthritis development and its timing in seropositive arthralgia patients. PD did not predict arthritis development, probably related to low PD frequency. A large study population needs to be screened to find only a small percentage of patients with US abnormalities, so expected use for routine clinical practice and to select individuals at risk of developing arthritis for preventive studies is low. However, based on our data we do expect that US can be of additional use for clinicians in those patients who have an intermediate risk of developing arthritis when calculating the prediction rule, as compared to those patients for whom the risk is more clearly defined based on clinical parameters (low and high risk).

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Supplementary Table 1. Association of ultrasound abnormalities with clinical arthritis development, ACPA positive versus negative patients (patient level)

US abnormalities	Arthritis yes	Arthritis no	OR (95% CI)	p-value
ACPA positive patients	n=42	n=49		
Synovial thickening* (16 joints)	16 (38%)	14 (29%)	1.5 (0.6-3.7)	p=0.4[†]
Synovial thickening (10 joints, no MTP)	8 (19%)	1 (2%)	11.3 (1.3-96)	p=0.01[‡]
Power Doppler* (16 joints)**	1 (2%)	3 (6%)	NA	NA
ACPA negative patients	n=9	n=63		
Synovial thickening (16 joints)	3 (33%)	16 (25%)	1.5 (0.3-6.6)	p=0.6[‡]
Synovial thickening (10 joints, no MTP)	2 (22%)	3 (5%)	5.7 (0.8-40)	p=0.1[‡]
Power Doppler (16 joints)**	1 (11%)	2 (3%)	NA	NA

[†] Chi-square test, [‡] Fisher's exact test.

* Results are presented for synovial thickening and Power Doppler in at least one joint

** Same results when excluding MTP joints

CI, confidence interval; MTP, metatarsophalangeal; OR, odds ratio; NA, not applicable (not calculated due to small numbers)

CHAPTER 11

Summary

The aim of this thesis "psychological and biological features influencing the risk for rheumatoid arthritis" was to further study the at-risk phase of rheumatoid arthritis (RA) by summarizing the literature (**Part I**) and producing new knowledge on symptoms (**Part II**), serological markers and imaging (**Part III**), with the ultimate goal of enhancing the prediction of future RA. The studies were performed making use of three sources: the prospective cohort of seropositive arthralgia patients from Reade, an international convenience sample of individuals at-risk of developing RA, and the Nivel Primary Care Database (Nivel-PCD).

PART I: REVIEWING THE AT-RISK PHASE OF RHEUMATOID ARTHRITIS

To have an overview of the literature on the at-risk phase of RA, but also learn about possible missing elements, the thesis starts with two reviews. The first review (**Chapter 2**) concluded that the risk for future RA development results from an interplay of genetic, reproductive and environmental factors. Therefore, prediction can be based on different characteristics as time progresses, e.g. in an early asymptomatic phase or a later symptomatic phase. The latter phase usually, but not always, is accompanied by autoimmunity. In the review eleven risk factor groups and five clinical prediction models that quantify the relative impact of the individual variables have been described. In the manuscript the conclusion was drawn that the clinical prediction rules all need validation and that RA at present should not be screened for outside of the research setting, because not all basic requirements for screening groups of people to predict a disease are satisfied. One of those requirements, the availability of a cost-effective intervention in the at-risk phase of RA, is discussed in the second review (**Chapter 3**).

The second review explains the not well described transition from early arthritis to established RA, the more or less abandoned concept of undifferentiated arthritis, and risk factors that affect these stages. Also, five prediction models for RA development that combine genetic with clinical and environmental factors are summarized. The review ends with investigating the possibility to prevent RA from occurring or from progressing to a more chronic stage. Hereby noting that sometimes joint inflammation at the stage of early arthritis may still resolve without further consequences or at least decrease to a barely detectable minimum without intervention. Possible options for the primary prevention of RA include life style interventions (dietary changes, stopping smoking, weight reduction), and dental care. No drug intervention has hitherto proven to be effective, and secondary prevention of undifferentiated arthritis progressing to RA with drugs is becoming less of an issue due to the high sensitivity of the 2010 ACR/EULAR criteria to already pick up patients with RA in early disease, and the tendency to treat early arthritis rapidly.

PART II: SYMPTOMS IN SEROPOSITIVE ARTHRALGIA PATIENTS AND IN PRIMARY CARE BEFORE ARTHRITIS DEVELOPMENT

To be able to integrate personal characteristics and symptoms in prediction models, one first needs to know what these characteristics/symptoms are and how frequently they occur in the at-risk phase of RA. In view of the vague and nonspecific first symptoms of many patients who later develop RA, it is also necessary to better characterize and measure these symptoms. Secondly, to be able to establish simple prediction aids in for example the general practitioner office, one needs to perform studies in primary care. These need to be large, since the prevalence of RA in the general population is low.

In **Chapter 4,** we longitudinally investigated the role of psychological symptoms and psychosocial vulnerability in arthritis development using 231 patients from the Reade seropositive arthralgia cohort. Higher scores for depressive mood and lower scores for social support were not associated with the development of arthritis nor with inflammation, as measured by the erythrocyte sedimentation rate. However, depressive mood was longitudinally associated with an increase in visual analogue scale (VAS) pain (regression coefficient (B) 2.34, 95% confidence interval (CI) 1.59 to 3.08, p<0.001), morning stiffness (B 4.09, CI 1.18 to 7.00, p<0.01) and tender joint count (rate ratio (RR) 1.06, CI 1.03 to 1.09, p<0.001). Lower social support was related to a higher VAS pain (B -1.97, 95% CI -2.77 to -1.17, p< 0.001), VAS morning stiffness (B -4.33, CI -7.40 to -1.28, *P* = 0.005) and tender joint count 53 (TJC53) (RR 0.93, 95% CI 0.91 to 0.96, p<0.001). No consistent associations were found between daily stressors, avoidance coping and the development of arthritis or other clinical parameters. The conclusion was that although no direct relation with arthritis development was found, clinicians should be aware of the fact that already in patients at risk of developing arthritis, depressive symptoms and low social support may increase musculoskeletal symptoms.

The presence and frequency of musculoskeletal and extra-articular symptoms, that were previously identified as relevant, were studied in **Chapter 5**. From an international convenience sample of individuals at-risk for RA, 219 individuals completed the newly designed Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire. The SPARRA questionnaire proved to have good psychometric properties, except for moderate construct validity and low responsiveness. The study showed that in individuals at risk of RA, all kinds of symptoms are frequent and severe, and have a high impact. Besides joint symptoms as was expected, this holds true also for general and nervous system-related symptoms such as burning and tingling sensations, numbness and fatigue, especially amongst anti-citrullinated protein antibody (ACPA) positive individuals.

In **Chapter 6**, a study of symptoms and diseases preceding inflammatory arthritis (IA) in primary care is reported on. To this end a nested case-control study was performed using Nivel Primary Care Database (Nivel-PCD). From Nivel-PCD, 2406 cases with a new diagnostic code of IA were selected, and 192 symptoms and diseases were investigated using the International Classification of Primary Care (ICPC-1) coding system. Compared

to control patients these cases had more musculoskeletal symptoms, infections, IA-related diseases and chronic diseases before the IA diagnosis. Specifically, the frequency of musculoskeletal symptoms increased in the final 1.5 years before IA diagnosis. Multivariable logistic regression analyses showed high odds ratios (ORs) (2.0 to 7.3) to predict IA for joint symptoms not otherwise specified, hand and wrist symptoms, chronic pain in the knee and carpal tunnel syndrome. Also, multiple comorbidities more frequently appeared in cases versus controls that mostly had not been reported previously in the pre-disease phase of IA. Main contributors were psoriasis, chronic enteritis/ulcerative colitis, gout, iron deficiency anemia, vitamin B12/folate deficiency anemia, asthma and diabetes mellitus. With the classification and regression trees (CART) methodology a structure using all investigated ICPC codes was made visible, resembling the way GP would detect patients to refer to the secondary health care system. Before use in clinical practice, however, these results need validation.

PART III: MARKERS FOR THE DEVELOPMENT OF RHEUMATOID ARTHRITIS

In the final part of the thesis a variety of markers was investigated. We aimed at expanding the evidence on blood markers as well as imaging.

Recently, serum 14-3-3η (eta) showed potential as a novel protein biomarker in predicting radiographic deterioration in early and advanced RA. In the study presented in **Chapter 7**, the association of baseline 14-3-3η with the development of clinically apparent arthritis was investigated in the Reade cohort of arthralgia patients positive for at least one serologic marker: rheumatoid factor (RF) or ACPA. Arthritis occurred in 43 (30%) of the 144 subjects and 14-3-3η was detectable up to 5 years before onset of clinical arthritis. 14-3-3η was present significantly more often (36% versus 14%, relative risk 2.5, CI 1.2 to 5.6, p=0.02) in those who developed arthritis compared with those who did not. Also, levels of 14-3-3η were higher in later arthritis patients (median 0.95 versus 0.28 ng/ml, p=0.02). 14-3-3η levels/positivity and ACPA, but not RF, were univariately associated with the development of arthritis. However, an incremental benefit of adding 14-3-3η after testing for RF and ACPA could not be demonstrated.

The previous chapter underscores the fact that RF and ACPA, especially when both are positive, are strong predictors for RA development, and therefore it is difficult for other biomarkers to add predictive value above these antibodies. Most studies investigated RF and ACPA at one time point or with multiple measurements in retrospective studies. In **Chapter 8**, the relation of RF and ACPA levels and their change over time in the period before arthritis development were investigated prospectively in the Reade cohort. A total of 983 antibody measurements were obtained from 263 patients, a median of 4 per person. Of these, 69 (26%) developed arthritis. Using joint models, it was found that ACPA was a good predictor of arthritis development, but the inclusion of time-updated measurements had limited additional predictive value over a baseline measurement. In these joint models, a linear mixed effects model of the development of RF and ACPA over time and a Cox-proportional hazards survival model for the arthritis development was

combined. The hazard ratio (HR) of the baseline ACPA was 2.6, and (counter to expectation) this only slightly increased to 2.8 when the time-updated ACPA levels were included. RF measurement had no predictive value for development of arthritis (HR baseline RF 1.1 and with time-updated values 1.2).

In **Chapter 9**, the predictive value of a novel marker to predict imminent onset of arthritis was replicated. Overall 28% of the individuals presenting with arthralgia and IgM rheumatoid factor and/or anti-CCP antibodies will develop RA within 3 years. Recent studies showed that among these RA-risk individuals risk for arthritis was 83% in the subgroup that had 5 or more dominant B-cell receptor (BCR) clones in peripheral blood (BCR positive individuals). In a prospective validation cohort study of 122 RA-risk individuals we showed that none of the BCR-negative RA-risk individuals developed arthritis, while 32 (73%) of the BCR-positive individuals did (estimated RR: 114.1; 95%-CI: 7.2 - 1819; $p<0.0001$). Among the BCR-positives 91% of the individuals with 9 or more dominant clones (n=22; 18%) developed arthritis within 3 years, after a median follow-up of 16 months (BCR-high positive group), while among individuals with 5 to 8 dominant clones 55% developed arthritis (BCR-median positive group; log-rank test $p=0.006$). We propose this BCR-test will support start of early intervention in BCR-high positive patients, supports retesting in BCR-medium positive patients, and may help rheumatologists to reassure BCR-negative individuals in an evidence-based way.

Finally, **Chapter 10** reports on an ultrasonography (US) study of a standard set of 16 joints in 163 at-risk patients. Of these patients, 51 (31%) developed clinical arthritis. US revealed synovial thickening and a Power Doppler (PD) signal in at least one joint in 49 (30%) and 7 (4%) of the patients, respectively. Synovial thickening was associated with both development and timing of clinical arthritis in any joint (the patient level), but only when metatarsophalangeal (MTP) joints were excluded from the US assessment (OR 6.6, CI 1.9 to 22, and hazard ratio 3.4, CI 1.6 to 6.8, respectively). It was also investigated whether specific subgroups could be identified in which the predictive capacity of US was highest. This was the case in patients with an intermediate to high risk of arthritis based on a prediction rule with clinical parameters.

CHAPTER 12

Discussion

The focus of this thesis was the use of symptoms and markers for the prediction of future rheumatoid arthritis (RA) or its proxy inflammatory arthritis (IA), building on earlier work in this field by us and others. Before one can use such markers for prediction and ultimately in clinical practice it is necessary to perform multiple steps of research. First, the target population needs to be determined[1]. In our case this is individuals at-risk for developing RA, which in itself is a difficult population to define. In addition, it is difficult to obtain large enough study populations as only 6% of RA patients are recognized as being at risk before the diagnosis[2]. Secondly, one should be able to accurately measure the markers that are the subject of investigation, so that they can be replicated and tested in future research on prediction of RA. For most serum markers cut-off values are now available. However, how would this be done for symptoms that in this at-risk phase usually are nonspecific? And thirdly, all these potential risk factors and their relative impact on the development of RA need to be integrated to enable meaningful future use in clinical practice.

Defining those at risk for developing rheumatoid arthritis

Delay in the assessment of patients with early arthritis is associated with more severe outcomes later on in the disease[3]. Also, the expectation that intervening in the at-risk phase of RA could be beneficial is based on the success of treatment of RA within 1 to 2 years after onset of clinical disease[4 5]. Therefore, a constant search for early predictors is subject of research. As RA has a low prevalence, around 0.5-1% in the general population, it would not be feasible to prospectively follow healthy individuals until they would develop RA. Therefore, mostly high risk populations are investigated, usually defined as either having auto-antibodies in the serum (RF and/or ACPA), having arthralgia or clinically suspect arthralgia (CSA) and/or being a first-degree relative of a patient with RA[6-8]. These studies however have the limitation that only selected groups of individuals were studied, usually after referral to secondary care because of more severe symptoms. Alternative study designs such as retrospective studies in blood donor cohorts or large registries from both primary as well as secondary health care overcome some of these limitations, although they themselves present with new limitations[9]. For example, the usually small cohorts in blood donor studies and the impossibility to perform a full chart review in large registry studies that sometimes makes the outcome more uncertain, especially in a disease such as RA which is a specialist diagnosis. In conclusion, a perfect study design to investigate the at-risk phase of RA remains elusive, and the best way to make progress in the prediction of RA is to combine study results from multiple designs.

Symptoms and the prediction of rheumatoid arthritis

When thinking of RA joint pain/swelling and morning stiffness are the first symptoms that come to mind. However, other symptoms may add value for its prediction, such as psychological factors including fatigue and depressive symptoms. Although in this thesis (Chapter 4) and a recent cohort study with clinically suspect arthralgia patients[10] no direct relation between psychological symptoms and vulnerability with regard to arthritis development was found, a recent publication using a population-based study design did conclude that having a major depressive disorder increases the risk of developing RA with 38%[11]. Multiple clinical prediction rules included (mostly joint) symptoms[12 13], but

in all the measured risk is a risk over a certain time period (usually the duration of the study period), which makes it an abstract figure for the individual person at risk. Also, the prediction rules all need to be validated before they can be used reliably in clinical practice. Therefore, more research on symptoms and their relation with arthritis prediction is warranted.

It is well known that symptoms associate with a variety of functional disabilities in patients at-risk of developing RA[14 15]. However, multiple questions arise on how to measure/quantify the underlying symptoms:

1. When to measure the symptoms? In this, the investigated population matters. To prevent later damage of RA it would be best to recognize its symptoms as early as possible[16]. New guidelines for general practioners (GPs) exist on which symptoms and signs are important[17 18]. The advice is to refer if any of the following are present: >3 swollen joints, metatarsophalangeal/metacarpophalangeal involvement, and/or morning stiffness of >30 minutes. Although these guidelines may help GPs in recognising RA, it would even be better that symptoms are recognised in an even earlier stage. Guidelines for this do not exist in general practice, and it would not be feasible to prospectively follow healthy individuals with or without particular symptoms until they would develop RA. Prediction including a time frame seems to become more feasible nearer to the onset of clinical RA, when the aspects of symptoms together with autoimmunity and inflammation can be taken into account. Another aspect of this discussion is that a delay exists in the assessment of patients with RA, and this delay comes from both patients and doctors[19]. It varies across countries depending on the structure of the health care system[20] and multiple reasons for delay have been assessed[21]. But still, no good way of preventing delay exists, especially in patients with a gradual development of symptoms.

2. How to measure the symptoms? A start was made in the study presented in Chapter 5 of this thesis. As a basis, patients with arthralgia and RA were asked to describe not only their symptoms, but were also asked in what way this symptoms could best be quantified and converted to a questionnaire[22 23]. This questionnaire was then used in a new population of individuals at risk of developing RA. However, whether this quantification is enough for good prediction of RA is part of ongoing investigation.

3. How to define symptoms that are specific for developing RA? A recent review stated that most symptoms occur well in advance of the RA diagnosis, but no validated screening tools containing only symptoms exist[24]. Besides, of all symptoms musculoskeletal pain contributes highly in general practice. In contrast with a relatively low prevalence of RA, these patients with musculoskeletal pain have a low chance of developing RA[25 26]. It was estimated that the diagnosis of IA (ICPC L88) was given to only 6 out of 400 patients with joint symptoms in general practice[27]. Despite this low prevalence, in Chapter 6 of this thesis multiple musculoskeletal symptoms were associated with development of IA, namely in the last 1.5 years before the diagnosis. These results need to be confirmed, but seem promising enough to support more research in the field of early detection of IA

patients in general practice. It is possible that this future research may be facilitated by large registries as presented in Chapter 6 and advanced statistical techniques for big data analysis. Also, large registries are more and more being combined with other sources such as biobanks, pharmacy data and in the future maybe even combined data from general practices and secondary/tertiary care.

Also, the concept of CSA, which for a large part includes symptoms, has been found to be predictive of RA development[28]. In an international study, forty-four (18%) of EULAR defined CSA patients developed arthritis and the risk of developing this arthritis was two-fold higher than in those not fulfilling the criteria[28]. It yielded a sensitivity of 84% and a positive predictive value (PPV) of 30% within 2 years. It should be noted that this predictive capacity is only that high in patient that were selected to have CSA by their rheumatologist, and was much lower if this selection by the rheumatologist did not occur. It has also been concluded that the symptomatic phase in CSA patient is different in those developing ACPA-positive RA compared to ACPA-negative RA[29].

Serological and imaging markers in the prediction of rheumatoid arthritis

RA emerges as the result of an interplay between genetic susceptibility and environmental factors, with immune dysregulation as an intermediate phenomenon[30-33]. In the literature, an abundance of markers that can be placed in one or more risk factor groups have been investigated. In this thesis, we aimed at expanding the evidence on serological markers as well as imaging. First, serum 14-3-3η (eta) was investigated. Although it showed a univariate association with arthritis development, it did not add predictive capacity above rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). This is most likely influenced by the selection method for this cohort (ACPA and/or RF positive, therefore the value of 14-3-3 in seronegative patients could not be ascertained). In other studies, 14-3-3η was found in synovial fluid of inflamed joints (not specifically of RA patients), facilitated the diagnosis of RA[34 35] and predicted outcome of RA[36-39]. Its role in the at-risk phase of RA needs to be further investigated.

Thereafter, we investigated the role of ACPA and RF levels over time, and found no added predictive value for arthritis development over baseline values. This was somewhat unexpected, since longitudinal studies of blood donors have shown rising concentrations of autoantibodies before RA[40 41]. To our knowledge, no other studies have reported on repeated measurements in prospectively followed patients at risk for RA. Our findings are in line with reports of stable autoantibody levels after the diagnosis of RA[42].

Thirdly, the number of dominant B-cell receptor (BCR) clones was higher in seropositive arthralgia patients that developed arthritis than in those who did not[43]. This highly predictive result was validated in a new cohort of at-risk patients in this thesis. This study also showed that a higher number of BCR clones was correlated with a higher arthritis risk. It appears that these BCR clones are present in the blood in the at-risk phase, then disappear from the blood during RA development and can then later be found in synovial fluid of inflamed joints[43].

Finally, synovial thickening in the hand joints (and not power doppler signal) on ultrasonography (US) was associated with both development and timing of clinical arthritis. Whether this finding is useful in clinical practice can be debated, as a large study group was necessary to find small differences, but specifically in patients in which the risk of developing RA is intermediate it might add information. Thus far, two other studies showed the additive value of US in the at-risk phase of RA, both in patients with an already high chance of developing RA[44 45]. In one of them US was part of a clinical prediction rule[45]. Another recent study showed an association of tenosynovitis in the digit flexor with RA development, but this study was also performed in an advanced stage, namely in those who already had early arthritis[46]. Several research questions on US imaging remain open[47 48].

Integration of risk factors for the prediction of rheumatoid arthritis

Pending the results of preventive interventions, there is an obvious need to improve prediction of RA at the individual level. This is exemplified by the fact that healthy first degree relatives (FDRs) of patients with RA would choose to take preventative medication if available[49]. The willingness to do this depended on the level of risk reduction (≥20 %) and the side effects of the medication (acceptable if the chance of serious adverse events was ≤10%). However, there are also concerns. For example, knowing the risk can cause FDRs to worry about other questions such as being unable to know the severity of the RA if it would emerge, stress about a possible future RA diagnosis and wanting to know the exact time of onset of this event[50]. Another question with an ethical dimension would be how long one should be treated to prevent RA. It is not known whether a short “reset” of the auto-immune system could suffice. On the other hand, long term treatment to prevent a disease that may never occur is difficult to accept, even without any side effects.

Further improvement of predictive ability may be expected from the integration of risk factors such as symptoms, serological and imaging markers. Up to now, several clinical and genetic prediction rules have been described, but none can accurately give the individual risk and especially the time of RA diagnosis[6 7]. Here, the BCR test with its very high predictive capacity, at least in the in the phase of seropositive arthralgia, may form an exception.

Future research should cover both the topics prediction and preventative treatment. Prediction can be enhanced by combining knowledge on existing risk factors, and by a better understanding of the pathogenesis of RA, which could then possibly lead to new predictive biomarkers.

In some diseases certain gene mutations can ascertain that the disease will develop. In RA, however, more than 100 loci have been described to be associated with RA, but odds ratios of developing the disease are usually low for one specific gene mutation. High contributors to the genetic risk are the HLA- DRB1 type, the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene and the peptidyl arginine deiminase type IV (PADI4) gene [51-53].

However, it is not part of clinical practice to measure these genes in individuals at risk for developing RA. Because of the low odds ratios it is not to be expected that newly found gene mutations will add much to the measurable genetic risk.

Another approach would be to look further into the cell types that contribute to the development of RA. The clinical phenotype RA may reflect many pathogenic pathways[30 53]. T and B cells have been implicated [53 54], interacting with macrophages and dendritic cells. Predictive biomarkers derived from activated immune cells include: the type 1 interferon signature[55], B-cell markers such as the B-cell signature[56] and above all B-cell receptor (BCR) clonal expansion[43]. It needs to be further established what the role is of these clones in the pathogenesis, and in which phase of the development of the disease they give the most information: is this already in the asymptomatic phase, or only shortly before the outbreak of clinical arthritis.

Finally, the needed accuracy of prediction also depends on the results of prevention trials. If e.g. the present APIPPRA trial of abatacept will show cost-effectiveness of the intervention, it could become a therapeutic option for persons fulfilling the inclusion criteria of that trial, i.e. those with a certain level of risk. It is an attractive prospect that those who could benefit from treatment in the at-risk phase, can be accurately detected by the BCR test. The next question is then what the advantage of treatment in the at-risk phase is (for those with a very high risk) versus postponing treatment until the phase of clinical arthritis. Apart from the lesser burden of active arthritis until reaching clinical remission, this could possibly be a higher chance of achieving drug-free remission through such very early treatment.

RESEARCH AGENDA

- Improve prediction models of RA by integrating personal characteristics, symptoms, and genetic information with new biomarkers.
- Establish simple prediction aids for different situations, for example for the general public, in the general practitioner office, or in the rheumatology clinic.
- Controlled intervention studies in persons at risk for RA in different stages

CONCLUSIONS

From the studies in this thesis the following conclusions can be drawn on symptomatology and markers for the development of future RA:

Symptoms

- Although depressive symptoms and low social support were not directly associated with arthritis development, they were associated with an increase of musculoskeletal symptoms.

- Musculoskeletal symptoms as well as extra-articular symptoms occur frequently in the at-risk phase of RA, and can be measured with the Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire.
- In individuals that later develop inflammatory arthritis (IA), musculoskeletal symptoms as well as infections, IA-related diseases and chronic diseases were more frequently recorded in primary care, compared to a control group. In particular, the frequency of musculoskeletal symptoms increased in the final 1.5 years before IA development.

Markers
- Although 14-3-3η was present significantly more often in those who developed arthritis compared with those who did not, it did not add additional predictive value over rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) measurement.
- The change of RF and ACPA levels over time did not add value over the baseline levels for predicting future arthritis.
- Presence of ≥ 5 dominant BCR clones is strongly associated with development of RA.
- Using ultrasonography (US), synovial thickening in the hand joints was associated with both development and timing of clinical arthritis. Its value seems highest in those with an intermediate or high risk of developing arthritis based on a prediction rule with clinical parameters.

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Appendices

NEDERLANDSE SAMENVATTING

Reumatoïde artritis

Reumatoïde artritis, ook wel RA genoemd, is een chronische ziekte waarbij er sprake is van ontstoken gewrichten, samengaand met pijn, zwelling en vaak ochtendstijfheid. De ziekte komt voor bij 0.5-1% van de (wereld)bevolking en is daarmee een van de meest voorkomende chronische ziekten. RA ontstaat meestal rond het 55ste levensjaar en komt vaker voor bij vrouwen. Indien de ontsteking niet snel behandeld wordt, kan gewrichtsschade ontstaan met als gevolg functionele beperkingen. Daarnaast kan RA gepaard gaan met vroegtijdig overlijden. Het is daarom belangrijk om de ziekte zo vroeg mogelijk te ontdekken. Er wordt dan ook steeds meer onderzoek gedaan naar het voorspellen van RA in een vroeg stadium.

Het ontstaan van reumatoïde artritis

Hoewel het voor een groot deel onbekend is hoe RA ontstaat, wordt er gedacht dat er sprake is van een wisselwerking tussen genetische aanleg, verstoring van het immuunsysteem en omgevingsfactoren. Een groot deel van de individuen die een verhoogd risico hebben op het ontwikkelen van RA gaat door een fase waarin er auto-immuniteit bestaat, gevolgd door lokale ontsteking die initieel nog niet duidelijk meetbaar is, en vervolgens gevolgd door een fase met klachten. Met auto-immuniteit wordt bedoeld dat er afweerstoffen worden geproduceerd die zich tegen het eigen lichaamsweefsel richten, waardoor ontstekingen kunnen ontstaan. Deze zogenaamde reuma-antistoffen zijn bijvoorbeeld reumafactoren (RF) en antistoffen tegen gecitrullineerde eiwitten (ook wel anti-CCP of ACPA genoemd), en komen bij een deel van de individuen voorafgaand aan RA voor. Hoe de antistofreactie tot stand komt is niet exact bekend, maar er wordt gedacht dat er sprake is van bepaalde triggers zoals een infectie of ontsteking van de mond, longen of darmen. Na ontwikkeling van de antistoffen komt een ontstekingsreactie in de gewrichten op gang, die zorgt voor gewrichtspijn (ook wel artralgie genoemd), maar ook voor algemene symptomen als vermoeidheid en ochtendstijfheid in het hele lichaam. Deze fase van symptomen kan maanden tot jaren duren.

Risicogroepen voor ontwikkeling van reumatoïde artritis

In theorie heeft iedereen in de algemene bevolking 0.5-1% kans op ontwikkeling van RA. Deze kans wordt echter sterk verhoogd indien er sprake is van een genetische aanleg (bijvoorbeeld in het geval van familieleden met RA), auto-antistoffen in het bloed (met name de genoemde RF en ACPA) of een bepaald klachtenpatroon. Uiteraard kan ook een combinatie van risicofactoren aanwezig zijn. Het klachtenpatroon bij een hoog risico patiënt wordt wel aangeduid met “clinically suspect arthralgia”, het gaat dan om gewrichtsklachten die door de reumatoloog verdacht worden bevonden voor een binnenkort optreden van RA. Belangrijke aspecten hierbij zijn: gewrichtspijn die ‘s ochtends erger is dan de rest van de dag, de aanwezigheid van ochtendstijfheid gedurende meer dan een uur en het aanwezig zijn van RA in de familie.

Onderzoek naar de fase voorafgaand aan RA wordt vaak gedaan in groepen mensen die voldoen aan 1 of meer van de risicofactoren. Hierbij is het goed om te realiseren dat de kans op ontwikkeling van RA wel verschilt tussen deze groepen. In het Reade onderzoekscohort, waarbij mensen met RF en/of ACPA antistoffen en daarnaast gewrichtsklachten worden gevolgd, is deze kans bijvoorbeeld 35% binnen 5 jaar. In een cohort dat gevolgd wordt in Leiden, bestaande uit individuen met "clinically suspect arthralgia", is deze kans 20%, en bij mensen met eerstegraads familieleden met RA zelfs maar 3.9%. Gezien de lage algemene kans op ontwikkeling van RA is het een uitdaging om een studie groep samen te stellen die groot genoeg is om verschillen aan te kunnen tonen tussen de individuen die wel en die geen RA ontwikkelen. Om deze reden wordt er gezocht naar alternatieve studie populaties, bijvoorbeeld grote databank studies waarbij bijvoorbeeld gegevens van nationale verzekerings-registraties worden gebruikt of gegevens uit huisartsenpraktijken.

Het voorspellen van reumatoïde artritis
Tot dusver bestaat er nog geen goed screeningsinstrument en bestaat er tevens nog geen behandeling om RA te voorkomen. Wel is er al veel onderzoek gedaan naar individuele risicofactoren, en is geprobeerd om deze risicofactoren te combineren in zogenaamde predictie modellen. Hierin wordt van elke risicofactor bepaald wat de bijdrage is aan het ontwikkelen van RA ten opzichte van de andere risicofactoren. Er zijn zowel klinische predictie modellen (deze bevatten gegevens als antistoffen, klachten en omgevingsfactoren) ontwikkeld als modellen waarin ook genetische factoren zijn meegenomen. Naar deze predictie modellen moet echter eerst meer onderzoek worden verricht voordat ze kunnen worden gebruikt in de dagelijkse praktijk.

Het risico op RA wordt voor ongeveer 65% bepaald door genetische achtergrond, zoals is vastgesteld in onderzoek met tweelingen. Hoewel al meer dan 100 genen zijn ontdekt die geassocieerd zijn met ontwikkeling op RA, verklaren deze genen tezamen slechts een klein deel (ongeveer 16%) van het ontstaan van RA. Genen die een grote invloed hebben zijn bijvoorbeeld meerdere delen van het HLA-DRB1 complex die ook wel de "shared epitope" wordt genoemd. Deze genen hebben een sterker effect bij rokers.

Naast roken lijken ook veel andere leefstijl en omgevingsfactoren van belang voor het tot uiting komen van RA. Dit zijn bijvoorbeeld ontsteking van het tandvlees, overgewicht, dieet (een Mediterraan dieet lijkt beschermend, eten van veel bewerkte producten juist niet) en blootstelling aan silica (chemische verbinding van metaal en zuurstof). Het gebruik van visolie, vitamine D en alcohol zouden juist beschermend kunnen werken. Roken is de grootste risicofactor in deze groep.

Andere risicofactoren zijn de antistoffen die reeds eerder werden genoemd. Naar RF en ACPA is het meeste onderzoek verricht. Ongeveer 2/3 van de RA patiënten test positief voor deze antistoffen, wat benadrukt hoe belangrijk de antistoffen zijn in onderzoek naar de fase voorafgaand aan RA. Antistoffen kunnen jaren voor het tot uiting komen van de ziekte aanwezig zijn in het bloed. Ze zijn echter niet bij iedereen aanwezig die RA ontwikkelt, en kunnen ook aanwezig zijn bij mensen die uiteindelijk helemaal geen reuma ontwikkelen.

Andere bloedeiwitten en afweercellen die een relatie met RA hebben zijn bijvoorbeeld anti-CarP antistoffen, type 1 interferon en B-cellen.

Tot slot wordt de laatste jaren steeds meer onderzoek verricht naar het gebruik van het klachtenpatroon van individuen met een verhoogd risico op het ontwikkelen van RA, voor het voorspellen hiervan. Symptomen zoals gewrichtspijn, zwelling en ochtendstijfheid zijn nodig voor het stellen van de diagnose, maar kunnen ook waardevolle informatie geven in de fase voorafgaand hieraan. Daarnaast kan het zijn dat symptomen die niet direct worden toegeschreven aan RA een voorspellende waarde hebben, zoals bijvoorbeeld vermoeidheid of depressieve gevoelens. Bij onderzoek naar symptomen komen echter ook een aantal vraagstukken naar voren: 1) Wanneer is het beste tijdstip om symptomen te meten; 2) Hoe meet men deze symptomen goed; en 3) Welke symptomen zijn specifiek voor RA en welke niet.

De in dit proefschrift genaamd "psychological and biological features influencing the risk for rheumatoid arthritis" gebruikte onderzoeks populaties

Studie populatie van Reade bestaande uit individuen met antistoffen en gewrichtsklachten
De studie werd opgezet in 2004 in Reade, een reumatologie en revalidatie centrum in Amsterdam West. De studie populatie bestaat uit individuen met een verhoogd risico op het ontwikkelen van RA doordat zij RF en/of anti-CCP antistoffen in het bloed hebben, in combinatie met het hebben van gewrichtsklachten. De studie werd opgezet om onderzoek te doen naar klinische factoren (zoals locatie van gewrichtsklachten) en serologische factoren (dat wil zeggen bepaalde stoffen in het bloed) die invloed hebben op de ontwikkeling van gewrichtsontsteking en die kunnen helpen bij het vroegtijdig voorspellen en opsporen hiervan. In de eerste jaren werd gevraagd of de deelnemers mee wilden doen in een geneesmiddelen onderzoek naar het effect van dexamethason (een ontstekingsremmer) op het voorkomen van RA. Deze medicatie bleek niet effectief, maar de studie werd voortgezet zonder medicatie en bevat inmiddels meer dan 600 deelnemers. Tijdens de studie worden de deelnemers jaarlijks gecontroleerd op klachten, bloedwaarden en het eventueel optreden van gewrichtsontsteking tot de studie periode van 5 jaar is voltooid. De studie populatie werd gebruikt in hoofdstukken 4, en 7-10 van dit proefschrift.

Internationale groep van individuen met een verhoogd risico op het ontwikkelen van RA
Hoewel meer dan 600 individuen in 1 cohort een groot aantal is, is het goed om naar een studie populatie te kijken die bestaat uit een verscheidenheid van individuen uit verschillende landen. Op deze manier zeggen de studie resultaten meer over individuen uit andere landen, en kan vervolg onderzoek makkelijker plaatsvinden. Ook kunnen de studie resultaten sneller worden verwacht, omdat het aantal personen in de studie sneller oploopt.

Voor hoofdstuk 5 werd data gecombineerd vanuit 5 Europese centra, waaronder het Reade cohort dat hierboven is beschreven. In Birmingham (Engeland) deden personen mee met "clinically suspect arthralgia" waarbij de antistoffen zowel positief als negatief konden zijn; in Stockholm (Zweden) deden individuen mee met gewrichtsklachten die anti-CCP antistoffen in hun bloed hadden; in Wenen (Oostenrijk) werden ook studie deelnemers met gewrichtsklachten gevraagd met of zonder antistoffen; en tot slot deden vanuit Geneve (Zwitserland) eerstegraads familie leden mee van patiënten met RA.

Nivel Zorgregistraties eerstelijn
Het Nivel is een onderzoeksinstelling in Utrecht. Nivel Zorgregistraties maakt gebruik van gegevens die routinematig in de zorg worden verzameld in zowel huisartspraktijken als apotheken. Voor hoofdstuk 6 werd gebruik gemaakt van de Nivel database waarin alle gegevens verzameld zijn. Deze bevat data van meer dan 1,5 miljoen Nederlandse inwoners, vanuit ongeveer 500 huisartspraktijken. Er worden gegevens verzameld over contact momenten door mensen bij de huisarts, ziektebeelden, medicatie voorschriften en bepaalde testen. Deze worden allen gecodeerd met een internationaal coderingssysteem (ICPC-1 codering).

Overzicht van de fase voorafgaand aan reumatoïde artritis (deel 1 van het proefschrift)
Hoofdstukken 2 en 3 bevatten overzichtsartikelen waarin alle aspecten betreffende de risico fase voorafgaand aan RA worden besproken. Hierin is aandacht voor zowel gegevens die de laatste jaren duidelijker zijn geworden, als elementen waar nog meer onderzoek naar nodig is. In hoofdstuk 2 wordt beschreven dat het risico op RA, zoals hierboven beschreven, bepaald wordt door een combinatie van genetische factoren, verstoring van het immuunsysteem en omgevingsfactoren. Al deze factoren kunnen gebruikt worden om RA in een eerder stadium te voorspellen, zowel in een fase dat er geen gewrichtsklachten bestaan als in een fase waarin dat wel zo is. In deze laatste fase met klachten komen vaak ook de antistoffen voor. In hoofdstuk 2 worden 11 risicofactor groepen benoemd, en 5 predictie modellen die factoren combineren. De conclusie werd getrokken dat er nog meer onderzoek gedaan moet worden naar deze predictie modellen voordat ze in de dagelijkse praktijk gebruikt kunnen worden, en dat er op dit moment nog geen goede mogelijkheden tot screening bestaan om RA aan te tonen. En zelfs al zou zo'n mogelijkheid bestaan dan nog zijn er een aantal voorwaarden waar aan voldaan moet worden om op grote schaal te gaan testen op een ziekte. Eén van die eisen zou zijn dat een ziekte met een relatief goedkope behandeling voorkomen kan worden. Ook dit is bij RA (nog) niet het geval. De pogingen om RA te voorkomen met zowel medicatie als leefstijladviezen zijn onderdeel van het tweede overzichtsartikel in hoofdstuk 3. Daarnaast wordt in dit tweede overzichtsartikel aandacht besteed aan de overgang van beginnende gewrichtsontsteking in weinig gewrichten naar gewrichtsontsteking die voldoet aan de classificatie criteria van RA.

Symptomen bij individuen met gewrichtsklachten en antistoffen, en in de huisartsenpraktijk, voorafgaand aan de ontwikkeling van gewrichtsontsteking (deel 2 van het proefschrift)

Voordat men persoonlijke kenmerken en symptomen kan meenemen in predictie modellen is het nodig om eerst onderzoek te doen naar welke karakteristieken en symptomen belangrijk zijn en hoe vaak ze voorkomen in de risico fase van RA ontwikkeling. Gezien deze klachten bij veel individuen in deze fase aspecifiek zijn is het ook nodig om deze symptomen beter te karakteriseren. Daarbij moet gerealiseerd worden dat studie groepen groot genoeg moeten zijn, omdat de frequentie van de RA diagnose (met name) in de huisartsenpraktijk laag is.

Gezien het feit dat depressie en RA vaak samengaan, werd in hoofdstuk 4 onderzoek verricht naar het effect van psychologische factoren en psychosociale kwetsbaarheid op de ontwikkeling van gewrichtsontsteking bij 231 individuen met gewrichtsklachten en antistoffen uit de Reade studie populatie. Er werd aangetoond dat een meer depressieve stemming en daarnaast minder sociale steun waren geassocieerd met meer pijnklachten, ochtendstijfheid en meer pijnlijke gewrichten bij onderzoek door de reumatoloog. Er werd geen directe relatie aangetoond met ontwikkeling van RA. Het lijkt echter belangrijk te zijn dat reumatologen naast vragen naar klachten van het bewegingsapparaat ook aandacht hebben voor psychologische factoren die voor de patiënten van belang kunnen zijn.

In hoofdstuk 5 werd door middel van een vragenlijst studie gekeken wat de frequentie van gewrichtsklachten en overige klachten (bijvoorbeeld vermoeidheid en concentratie problemen) was bij individuen in de risicofase voorafgaand aan RA. Deze studie werd uitgevoerd in Europees samenwerkingsverband (zie hierboven). De vragenlijst, genaamd de “Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA)” vragenlijst, werd gemaakt op basis van interviews over klachtenpatronen met individuen die de reumatoloog bezochten in verband met gewrichtsklachten en antistoffen, of die al de diagnose RA hadden gekregen. In een nieuwe studie groep, bestaande uit 219 deelnemers, werd aangetoond aan dat de SPARRA vragenlijst betrouwbare gegevens produceert en dat een groot deel van de deelnemers forse symptomen heeft met een hoge frequentie. Behalve de verwachte gewrichtsklachten als pijn en stijfheid, kwamen hierbij ook algemene symptomen als branderige en tintelende gevoelens, doofheid en vermoeidheid veel voor. Dit was met name het geval bij de mensen die anti-CCP antistoffen in hun bloed hadden.

In hoofdstuk 6 werd onderzoek gedaan naar de risicofase van RA, of eigenlijk in bredere zin inflammatoire ontstekingsziekten, aan de hand van gegevens uit de huisartsenpraktijk. Er werden 2406 patiënten geselecteerd die de diagnose inflammatoire gewrichtsontsteking hadden gekregen, en hierbij werden controle patiënten (2 voor elke patiënt) gezocht zonder deze diagnose. Onder de code voor inflammatoire gewrichtsontsteking vallen naast RA ook de ziekte van Bechterew (ontstekingsziekte van het bekken en de wervelkolom) en artritis psoriatica (gewrichtsontsteking bij de huidziekte psoriasis). Vervolgens werd gekeken of bij de mensen voorafgaand aan de diagnose bepaalde gewrichtsklachten, infecties of

andere ziekten vaker voorkomen dan bij controle patiënten. Dit bleek inderdaad het geval te zijn, waarbij dit het duidelijkst was voor gewrichtsklachten. Deze namen sterk toe in de laatste 1,5 jaar voorafgaand aan de diagnose, terwijl de frequentie bij controle patiënten stabiel bleef over de tijd. Ook infecties en andere ziekten kwamen vaker voor bij patiënten ten opzichte van controles, wat over de gehele studie periode van 6 jaar leek te bestaan. Hoewel de studie resultaten nog moeten worden bevestigd in ander onderzoek, lijkt het zo te zijn dat de huisarts alert moet zijn als mensen met de volgende klachten/ziekten zich ook presenteren met gewrichtsklachten: chronische pijn in de knie, het carpaal tunnel syndroom (pijn in de hand door beklemming van een zenuw), bloedarmoede met ijzer tekort, een vitamine B12 of foliumzuur tekort, astma of suikerziekte.

Overige risicofactoren voor ontwikkeling van reumatoïde artritis (deel 3 van het proefschrift)

In het laatste deel van het proefschrift worden een aantal risicofactoren onderzocht, waarbij zowel wordt gekeken naar risicofactoren gemeten met bloedmonsters als onderzoek met echografie.

Recent onderzoek toonde aan dat het eiwit 14-3-3η in het bloed een goede voorspeller was voor achteruitgang op röntgenfoto's (meer zichtbare schade van de botten) bij mensen met zowel beginnende als gevorderde RA. In hoofdstuk 7 werd gekeken of dit eiwit 14-3-3η ook het ontstaan van gewrichtsontsteking kon voorspellen bij mensen met gewrichtsklachten die RF of anti-CCP positief zijn. De conclusie was dat aanwezigheid van 14-3-3η en de hoogte ervan beide geassocieerd waren met ontwikkeling van gewrichtsontsteking. Echter was het wel zo dat er bij correctie voor RF en anti-CCP geen duidelijke relatie meer werd gevonden, wat betekent dat het gevonden effect mogelijk meer een gevolg was van de RF en anti-CCP waarden dan van 14-3-3η.

In het vorige hoofdstuk werd gezien dat RF en anti-CCP belangrijke voorspellers zijn voor ontwikkeling van RA. In hoofdstuk 8 deden we onderzoek naar verandering van de hoogte van deze antistoffen over de tijd. Over een studie periode van 5 jaar werden per patiënt meerdere bloedmonsters onderzocht. Echter, de verwachting dat de hoogte van antistoffen over de tijd meer voorspellende waarde zou hebben dan een op zichzelf staande meting bleek niet uit het onderzoek. Er bestond slechts een beperkte toegevoegde waarde van het meermaals meten van de antistoffen in de loop van de tijd.

Eerder onderzoek toonde aan dat B cellen (bepaalde afweercellen) belangrijk zijn bij het ontstaan van RA. Dominante B cel receptor (BCR) klonen bleken daarbij een sterke voorspeller van de ziekte. Dit resultaat werd in hoofdstuk 9 bevestigd in een nieuwe studie groep. Met behulp van een bloedtest werd gekeken of de aanwezigheid en het aantal van deze BCR klonen toeneemt bij individuen die uiteindelijk RA ontwikkelen ten opzichte van degenen waarbij dit niet het geval was. Er bleek een duidelijk verschil te bestaan en de toekomst zal moeten uitwijzen hoe dit kan worden gebruikt in de dagelijkse praktijk van de reumatoloog.

Tot slot werd in hoofdstuk 10 onderzoek gedaan naar de rol van echografie. Er werd een standaard set van 16 gewrichten gescand waarbij gekeken werd naar ontsteking in het gewricht (ook wel synoviale verdikking genoemd) en de doorbloeding in het gewricht (gemeten met power doppler). Er werd gezien dat het maken van een echo van de voetgewrichten geen onderscheid maakt tussen degenen die gewrichtsontsteking ontwikkelen en degenen die dat niet doen. Ontsteking op een echo van de handgewrichten was wel geassocieerd met ontwikkeling van gewrichtsontsteking en ook met de timing van deze ontsteking. Het is echter wel zo dat er een groot aantal personen een echo moet ondergaan om slechts bij een klein deel echo afwijkingen te vinden. Het is dus de vraag of het zinvol is om bij iedereen die een verhoogd risico op RA heeft een echo te maken. Het lijkt wel duidelijk bij te dragen bij patiënten waarbij het risico op RA nog onduidelijk is op basis van klinische kenmerken.

Conclusie

Vanuit de studies in dit proefschrift vallen de volgende conclusies te trekken over symptomen en overige risicofactoren voor ontwikkeling van RA in de toekomst:

1. Symptomen

- De aanwezigheid van depressieve klachten en lage sociale steun waren niet direct geassocieerd met ontwikkeling van gewrichtsontsteking, maar wel met een toename van gewrichtsklachten.
- Zowel gewrichts- en spierklachten als symptomen buiten het gewricht komen vaak voor in de risicofase voorafgaand aan RA, en deze symptomen kunnen betrouwbaar gemeten worden met de “Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA)” vragenlijst.
- Bij individuen die later gewrichtsontsteking ontwikkelen komen gewrichtsklachten, infecties, en aan RA-gelinkte ziektebeelden gemeten met gegevens uit de huisartsenpraktijk vaker voor dan bij een controle groep. Met name de gewrichtsklachten nemen toe in de laatste 1,5 jaar voor de diagnose.

2. Overige risicofactoren

- Het eiwit 14-3-3η was vaker aanwezig bij degenen die gewrichtsontsteking ontwikkelen dan bij degenen waarbij dit niet het geval was. Er bestond echter geen toegevoegde waarde bij de voorspelling van RA boven de antistoffen RF en anti-CCP.
- Het meerdere malen meten van RF en anti-CCP levels over de tijd had geen toegevoegde waarde boven het eenmaal meten van deze antistoffen bij het voorspellen van RA.
- Aanwezigheid van BCR klonen is sterk geassocieerd met ontwikkeling van RA.
- Het hebben van gewrichtsontsteking in de handgewrichten gemeten met echo is geassocieerd met zowel de ontwikkeling als de timing van gewrichtsontsteking zoals gemeten door de reumatoloog. De waarde van echo lijkt het hoogst in de groep van individuen waarbij het risico op RA op klinische gronden alleen onzeker is.

PHD PORTFOLIO

Name PhD student: M.H. van Beers-Tas

PhD period: July 2013 - July 2017 Reade,
detached one day a week to the NIVEL research institute Utrecht from December 2015 - July 2017

Name PhD supervisor: Prof. dr. D. van Schaardenburg

General courses		
Master Evidence Based Practice - AMC/UvA	2012-2014	97 ECTS
OpenClinica training (database cursus) - VUmc	2015	0.5 ECTS
EULAR on-line course in Paediatric Rheumatology	2015-2016	3 ECTS
The AMC World of Science - AMC	2017	0.7 ECTS
Basis cursus Regelgeving en Organisatie Klinisch Wetenschappelijk Onderzoek (BROK) - AMC	2017	0.9 ECTS
Specific courses		
Advanced Topics in Biostatistics cursus - AMC/UvA (deels): *joint models, longitudinal data analysis, correlated data, missing data*	2014	1 ECTS
Statistical courses - Reade/VUmc *longitudinale data analyse 1 & 2, cluster analyse, multi level analyse*	2017	0.4 ECTS
Cursus onderwijs vaardigheden - HvA *algemene onderwijs principes, presentatie van onderzoeksresultaten, vertaling onderzoeksresultaten in een leermodule (bijv. elearnings), verdieping en combinatie van bovenstaande onderwerpen*	2016-2017	0.4 ECTS
Seminars and workshops		
Weekly department rheumatology and research seminars	2013-2017	5 ECTS
Investigator meeting, APIPPRA trial, King's College London	2014	0.25 ECTS
Moderator of a 1 day international science meeting	2016	0.25 ECTS

Presentations		
Serum 14-3-3eta predicts the risk of RA development and its higher titres are associated with higher risk. EULAR, poster presentation	2014	0.5 ECTS
Can autoantibody level changes predict arthritis in arthralgia patients? EULAR, poster tour	2015	0.5 ECTS
Helpen herhaalde metingen van autoantistoffen voor de predictie van artritis bij artralgie patiënten? NVR, poster presentation	2015	0.5 ECTS
Early detection of inflammatory arthritis: the role of musculoskeletal symptoms, infections and rheumatoid arthritis-related comorbidities in primary care. EULAR, poster presentation	2016	0.5 ECTS
First report of symptoms using the symptoms in persons at risk of rheumatoid arthritis (SPARRA) questionnaire. EULAR, poster presentation	2016	0.5 ECTS
Initial validation of the symptoms in persons at risk of rheumatoid arthritis (SPARRA) questionnaire. EULAR, poster presentation	2016	0.5 ECTS
Early detection of inflammatory arthritis: the role of musculoskeletal symptoms, infections and rheumatoid arthritis-related comorbidities in primary care. NVR, oral presentation	2016	0.5 ECTS
First report of symptoms using the Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire. NVR, oral presentation	2016	0.5 ECTS
Early detection of inflammatory arthritis: the role of musculoskeletal symptoms, infections and rheumatoid arthritis-related comorbidities in primary care. Amstel symposium, oral presentation	2017	0.5 ECTS
The Symptoms in Persons At Risk of Rheumatoid Arthritis (SPARRA) questionnaire: validation and results. EULAR, oral presentation	2017	0.5 ECTS
Conferences		
NVR (Nederlandse Vereniging voor Reumatologie) najaarsdagen in Arnhem, the Netherlands	2013	0.5 ECTS
NVR (Nederlandse Vereniging voor Reumatologie) najaarsdagen in Arnhem, the Netherlands	2014	0.5 ECTS
EULAR (EUropean League Against Rheumatism) congress in Paris	2014	1 ECTS
EULAR (EUropean League Against Rheumatism) congress in Rome	2015	1 ECTS
ACR (American College of Rheumatology) congress in San Francisco	2015	1.25 ECTS
NVR (Nederlandse Vereniging voor Reumatologie) najaarsdagen in Arnhem, the Netherlands	2016	0.5 ECTS
Amstel symposium, AMC, Amsterdam	2017	0.25 ECTS
EULAR (EUropean League Against Rheumatism) congress in Madrid	2017	1 ECTS

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1. Rheumatology:

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CURRICULUM VITAE

Maria Helena Tas (roepnaam Marian) werd op 15 maart 1986 geboren in de Stolpen, een klein “dorpje” in de kop van Noord-Holland. Ze groeide op in ‘t Zand, en behaalde op achttienjarige leeftijd haar VWO atheneum diploma aan de Gemeenschappelijke Scholen Gemeenschap in Schagen. Van 2004 t/m 2010 studeerde ze geneeskunde aan de Universiteit Utrecht, waarbij de propedeuse cum laude werd afgesloten. Hierna volgde een ANIOS periode kindergeneeskunde in het Sint Antonius ziekenhuis in Nieuwegein. Deze periode werd op 23 juni 2012 afgesloten met een speciale gebeurtenis. Ze trouwde met Joram Meda van Beers, waarna haar naam veranderde in Marian H. van Beers-Tas. Na een periode van zoeken naar een promotie plek en het schrijven van een (helaas mislukte) subsidie aanvraag, samengaand met werken bij de jeugdgezondheidszorg bij de GGD Zaanstreek Waterland, kon zij op 15 juli 2013 beginnen aan het promotie traject welke leide tot het huidige proefschrift. Dit onderzoek vond plaats bij Reade in Amsterdam, centrum voor revalidatie en reumatologie, op dit moment aangesloten bij het Amsterdam Rheumatology and immunology Center (een samenwerkingsverband met het Amsterdam UMC, locaties AMC en VUmc). Tussendoor werd het gezin uitgebreid met Luka Meda van Beers (19 februari 2014) en Bente Anna Maria van Beers (9 mei 2016), en verruilde het gezin een bovenwoning in Amsterdam voor meer ruimte in Kudelstaart. Daarnaast sloot zij op 9 februari 2015 de opleiding tot klinisch epidemiloog (masteropleiding Evidence Based Practice) aan de Universiteit van Amsterdam af. Vanaf 1 september 2017 is ze werkzaam als ANIOS kindergeneeskunde in de Noordwest Ziekenhuigroep locaties Alkmaar en Den Helder.