Predictive factors for outcome of rheumatoid arthritis

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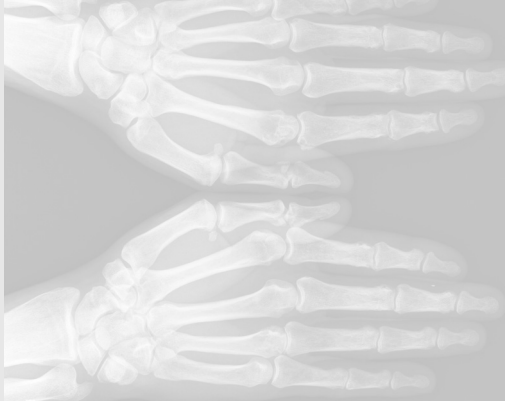

Predictive Factors for Outcome of Rheumatoid Arthritis

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Cover: X-rays of hands and feet from a RA patient showing erosions and joint space narrowing.

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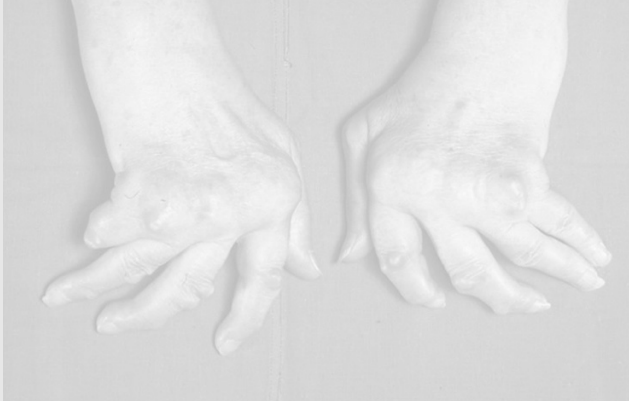

CHAPTER 1

General Introduction

RHEUMATOID ARTHRITIS

Although records of diseases with features mimicking those of rheumatoid arthritis have been around as early as the prehistoric ages, the term rheumatoid arthritis has been introduced around the middle of the 19th century. Initially starting as an ill-defined and rather underrated clinical image, it took until the 20th century during which it established its status as a full-grown disease.[1] Especially in the last few decades, the scientific progresses went rapidly and many new insights have been gained in aspects of its etiology and pathophysiology.[2]

Nowadays rheumatoid arthritis (RA) is a disease that is recognized as a major inflammatory arthritis of the joints that, can be found in approximately 1% of the population worldwide. The inflammation is characterized by a symmetric and poly-articular distribution that primarily affects the synovium of the small joints of hands and feet and can lead to subsequent localized joint destruction. It is considered to have an autoimmune origin because of the presence of self-reactive antibodies, such as anti-citrullinated protein antibodies (ACPA), thereby reflecting the complexity of the disease (Figure 1).[3,4] If left unattended or not properly treated, RA can lead to increased disability or even invalidity of patients in their normal daily functions, thereby reducing the quality of life. For society, this can ultimately lead to enormous costs in healthcare and loss in workforce.[5-9]

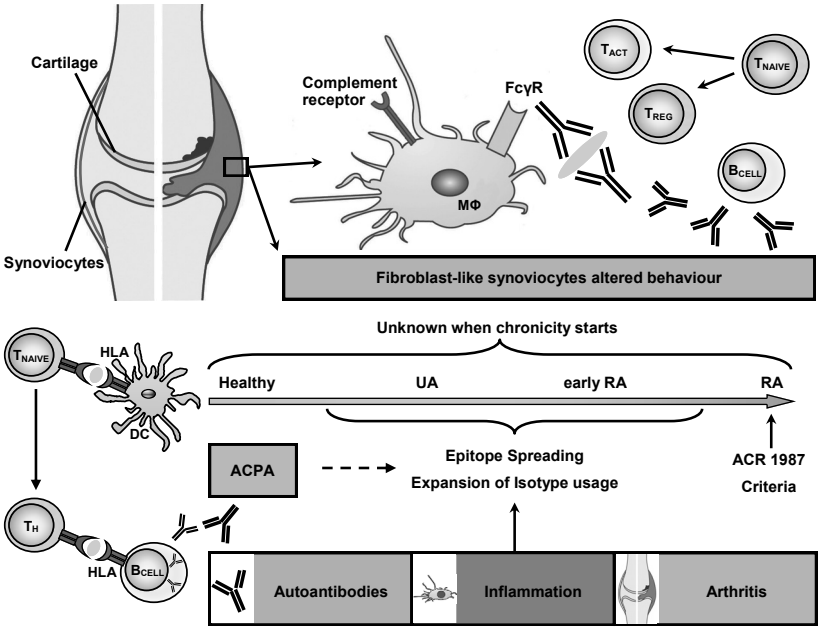

Figure 1. Overview of the complex nature of RA. FcγR: Fc-γ receptor; MΦ: macrophage; T_{ACT}: activated T-cell; T_{REG}: regulatory T-cell; HLA: human leukocyte antigen; T_H: helper T-cell; ACPA: anti-citrullinated protein antibodies; UA: undifferentiated arthritis; RA: rheumatoid arthritis; ACR: American College of Rheumatology. Adapted from Scott et al[4]

CLASSIFICATION AND DEVELOPMENT OF RA

In order to achieve early recognition of patients at risk, the 1987 American College of Rheumatism (ACR) classification criteria for RA[10] and more recently the revised 2010 classification criteria for RA, a joint initiative of the ACR and the EUropean League Against Rheumatism (EULAR),[11] have been developed (Figure 2). These sets of classification criteria, although not devised as diagnostic criteria, have been and will be frequently used to identify RA patients, both with established RA (1987 ACR) and with the intention of indentifying patients in a very early stage of RA (2010 ACR/EULAR).

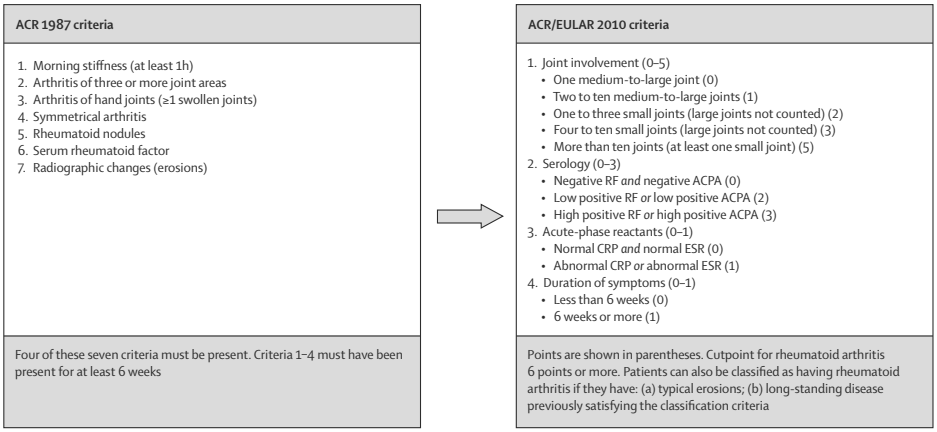

Figure 2. Overview of the 1987 ACR and the 2010 ACR/EULAR criteria. Adapted from Scott et al[4]

RA can develop from undifferentiated arthritis (UA), which is defined as having a form of arthritis not fulfilling the criteria for RA or for any other rheumatologic disease. From the Leiden Early Arthritis Clinic (EAC), a prospective inception cohort, it is known that about 40% of the patients, initially diagnosed with UA, will eventually progress to RA (Figure 3).[12,13]

RA is characterized by an insidious onset combined with slow or rapid progression and frequently a severe outcome (Figure 3).[14] Cumulative evidence, indicating that a delay in treatment leads to worse outcome,[15] together with the increased availability and performance of newer and more aggressive treatments,[16-19] make it exceptionally valuable to aim for early intervention of patients diagnosed with RA. Application of these modern treatments however should be done with caution to prevent overtreatment of less severe patients and associated detrimental short and long-term side effects.[20-23]

PREDICTION OF DISEASE OUTCOME IN RA

The ultimate goal in the treatment of RA patients would be the ability to predict the individual patients' chance of developing RA and the disease course of RA and subsequently apply a per-

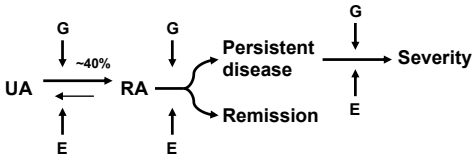

Figure 3. Model of the factors involved in the development and outcome of RA. UA: undifferentiated arthritis; RA: rheumatoid arthritis; G: genes; E: environment

sonalized treatment.[24] However, the amount of people affected and the course of severity of the disease as described by epidemiological data, show that both on a population level as well as on the level of the individual patient the disease varies in outcome and presentation. Evidence suggests that environmental, genetic as well as serologic factors influence not only the development of RA but also its severity, either resulting in persistent disease or, preferably, remission (Figure 3). However, the precise contribution of these factors for the different disease outcomes has yet to be unraveled.[25] Together with already known factors, newer risk factors yet to be discovered may lead to the identification of new pathways, and may ultimately contribute to the development of patient tailored treatment therapies.[26-28]

The role of genetics

Numerous efforts to better understand and further define the role of genetics in the development and disease course of RA have resulted in enormous progression during the last decade.[29,30] Analyzing variations in genetic constitution between patients and healthy controls has led the way to the discovery of specific genetic variants that show an association with a higher risk on developing RA. To determine these new genetic variants, referred to as so-called single nucleotide polymorphisms (SNPs), two different methods have been used for genotyping. The first method is the candidate gene approach, implicating that based on *a priori* knowledge of disturbances in function or homology to other diseases, the corresponding genetic regions are selectively targeted for analysis. The second method is the genome wide association study (GWAS) that is an unbiased approach that scans the whole genome. Until now this has resulted in the identification of over thirty genetic regions that associated with development of RA.[31-40] In contrast to the susceptibility to RA however, the influence of genetics on the severity of the disease course in RA remains (fairly) unknown, since only relatively few studies have thus far shown an association between a genetic variant and the disease course.[41-44] Moreover, these studies are all single data and were not replicated to confirm their findings, thereby leaving questions about the role of genetics on the severity of RA largely unanswered.

The role of serology

Serology comprises a second group of factors that are in the center of attention, both for further understanding RA as well as the use for predicting its development and outcome. These factors,

representing several pathways that are involved the pathophysiological processes underlying RA and its phenotypical appearance, can be measured in the serum of RA patients.[45]

One of the key elements of RA is inflammation. In the clinic, inflammation of the joints is objectified by quantifying the joint swelling of patients using a swollen joint count (SJC). The SJC is correlated with the serum levels of systemic inflammatory markers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) that reflect inflammatory burden in RA.[46] Other serum markers may represent more localized inflammatory processes with pro-inflammatory and regulatory functions in the rheumatic joint. As schematically presented in Figure 4, markers like interleukins (ILs), tumor necrosis factor (TNF) and interferon gamma (IFN-γ), so-called cytokines, reflect the interplay between the cells of the immune system involved in the localized inflammation of the joint space, and the cellular composition of the cells that produce them, e.g. B- and T-lymphocytes cells, macrophages and fibroblast like synoviocytes.[47-50] These factors and their corresponding cellular components are known not only to initiate disease processes, but also to maintain the inflammatory reaction. Subsequently, this may cause disturbances in the homeostasis of cartilage and bone that can ultimately result in joint damage.[50]

A special element of serology is formed by the presence of autoantibodies. Although the exact mechanisms are still unclear, the scientific view on the pathophysiological basis for RA has changed enormously since the concept of "immune hyper-reactivity" emerged in the mid-20th

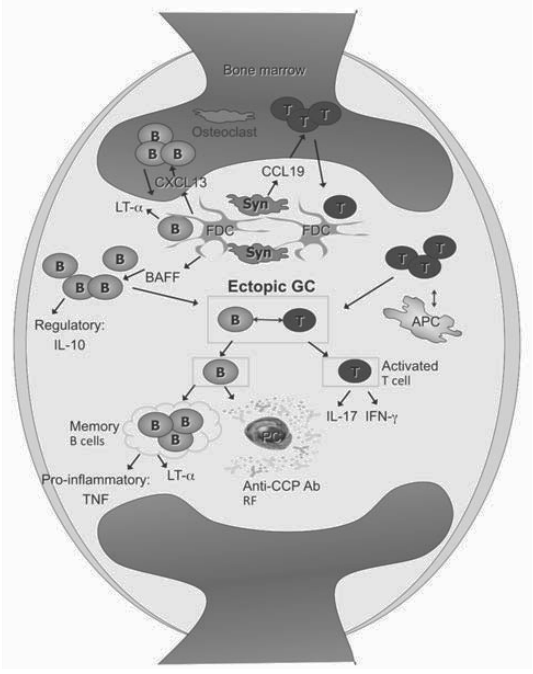

Figure 4. Schematic overview of the inflammatory processes in the rheumatic joint. T: T-lymphocyte; B: B-lymphocyte; Syn: synoviocyte; FDC: follicular dendritic cell; GC: germinal center; APC: antigen presenting cell. Adapted from Marston et al[50]

century. Reflecting its status of a disease with an autoimmune origin, the classical rheumatoid factor (RF) and the more recently discovered antibodies directed against anti-citrullinated proteins (ACPA)[51] can be detected in the serum of patients (Figure 5). The presence of both is associated with an aggressive and destructive disease course.[3,52]

Taken together, serological factors are thus involved in the various pathophysiological processes that take place in RA and as such would represent possible targets for developing newer treatment therapies. In addition, they can be used for predicting the development as well as disease outcomes of RA. The degree in which these factors are in fact useful as a marker have been the subject of discussion, and has led to the development of a set of criteria that has to be fulfilled for a factor to be regarded as a real 'biomarker'.[53]

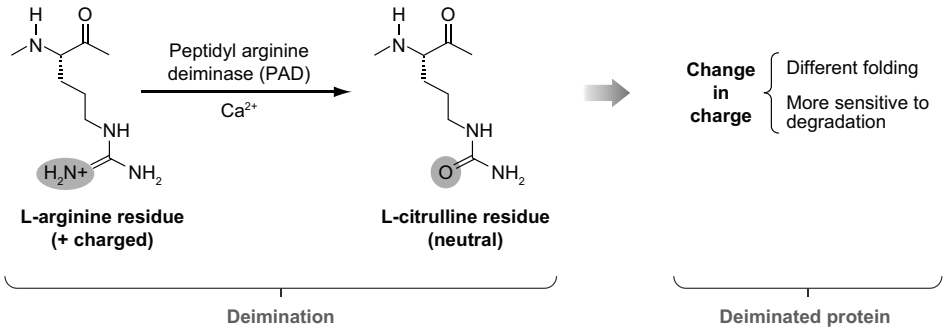

Figure 5. The process of citrullination. Citrullination is an enzymatic conversion that results in the loss of one positive charge for every arginine residue converted to a neutral citrulline, by deimination of peptidylarginine to peptidylcitrulline by the enzyme peptidyl arginine deiminase (PAD). The change in charge causes changes in intra- and intermolecular interactions, which could lead to altered protein folding, enhanced degradation by proteases, and exposure of cryptic epitopes. Adapted from Klareskog et al[51]

The role of environmental factors

In addition to genetic factors and serology, a third group of factors shown to associate with the development and disease course of RA are environmental factors. Several environmental factors have suggested potential candidates to influence RA.[54] Thus far, smoking is regarded as the most important environmental risk factor in the ACPA-positive subset of RA patients.[55-57] However, for other environmental risk factors, like alcohol, socioeconomic status and region of birth, the effect on RA is less well defined. In addition, also infectious agents could have a possible role in the activation of immune responses as observed in RA.[58]

Explanatory properties of predictive factors

Thus, although in the last decade clearly huge progress has been made with the identification of numerous genetic, serological and environmental factors that show an association with the development and severity of RA, together they do not completely explain the development and outcome of RA. It is recognized that genetics explain only ~50% of the susceptibility to RA.[59]

For the severity of RA, the exact contribution of genetics is not defined yet. In summary, the complexity of RA is illustrated by the observed interplay between several factors and the limited explained variance of the risk factors that are known thus far.

OUTCOME MEASURES

In order to identify new risk factors, different outcome measures can be used for evaluating the predictive performance for the development and disease course of RA.

For predicting outcome in UA, fulfillment of the criteria for RA can be determined. Other outcome measures that are frequently used are the initiation of therapy and the development of persistent disease.[11]

For investigating the severity of the disease course of RA, also various outcome measures thus have far been used. These concern "clinical factors" that are used to assess the reaction to treatment like disease activity score (DAS), health assessment questionnaire (HAQ) and joint swelling,[60] or laboratory measures like CRP and ESR. An objective measure for evaluating the course of RA is the development and progression of radiographic joint damage in the hands and feet. This can be measured by validated methods and is associated with inflammation and disability.[61,62] Other outcome measures that are used (less frequently) are also the achievement of remission and, although somewhat controversial, repair of erosions.

Radiographic joint damage

For assessing the amount of joint destruction that is visible on radiographs several methods exist. Compared to the Larsen and the Ratingen scoring methods, the Sharp/van der Heijde method is considered as the most sensitive method for measuring joint destruction.[63] In an observational cohort study scoring is done chronologically, thus with known time order.[64] The Sharp/van der Heijde score consists of a measure for cartilage degradation, the joint space narrowing (JSN), and a component reflecting the amount of bone degradation, the erosion score (ES). Taken together, they are referred to as the total Sharp/van der Heijde score (SHS), which will be used as the main outcome measure in this thesis.[65]

Evaluation of radiographic data is in literature frequently performed in a cross-sectional way, on single time points. Van der Helm-van Mil et al point out that ideally, joint damage is measured repeatedly over time as this may lead to a more precise estimation of an individual's progression rate.[62]

Achievement of (clinical) remission

A second outcome measure to evaluate the effect of risk factors on the outcome of RA is the achievement of remission. Remission as a clinical endpoint in studies has been used since many years.[66,67] The achievement of remission is self-evidently the most favorable and desired disease outcome,[68] but only ~10-15% of patients were observed to reach this goal.[69] Although different

definitions have been used over the years, to achieve disease-modifying antirheumatic drugs (DMARD)-free remission as it is has been used in this thesis, a patient has to fulfill three criteria: 1) no current use of DMARDs, 2) no swollen joints, and 3) classification as DMARD-free remission by the patient's rheumatologist.[69]

Repair of joint erosions

A somewhat controversial and less well documented outcome measure is the occurrence of repair of bone erosions. These sites of repair are characterized by the formation of new bone leading to a reduction in the magnitude of the previously developed sites of erosion (Figure 6).[70] The characteristics of repair as well as the factors responsible for this shift in balance from bone degradation to bone formation however are not clear yet. Identifying these factors could help both in elucidating the causal pathophysiological mechanisms of RA and developing treatment targets for inducing the mechanisms of repair of erosions.

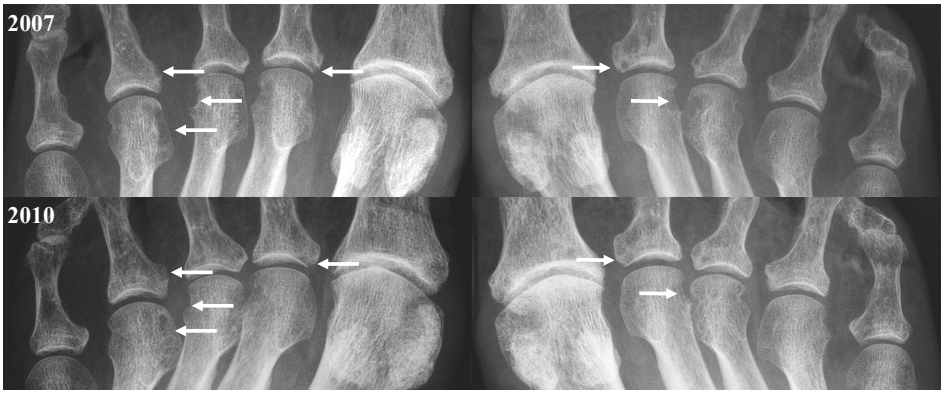

Figure 6. Example of repair of joint erosions. The arrows indicate sites of erosions in the year 2007 in the upper panel. Subsequently, in 2010 shown in the lower panel, these sites have been the subject of new bone formation, so-called repair

OUTLINE OF THIS THESIS

Risk prediction in RA is in the center of attention, with the development of personalized medicine as the final endpoint. Especially during the last decade, enormous advances in this field have been achieved, but mainly in identifying risk factors for the risk prediction of RA susceptibility. Risk factors for the outcome of RA, however, have thus far been scarcely explored.

The aim of this thesis was to study the risk prediction in RA, with the main focus on the disease outcome of RA and to lesser extent the development of RA from UA. For this purpose, longitudinal radiograph data from RA patients that were enrolled in the Leiden Early Arthritis Cohort between 1993 and 2006,[12] an inception cohort that has been up and running for a period

of 17 years, were collected and scored using the Sharp/van der Heijde method. Together with other outcome measures, these data were subsequently used for risk estimation.

In the **first part,** in **chapter 2** of this thesis, a detailed explanation how to analyze longitudinal radiological data is provided. It describes and compares different statistical methods of analysis and their advantages and disadvantages given the presence of repeated measurements. Detailed information is given about the development of the model referred to as the repeated measurement analysis (RMA). This model is one of the cornerstones in this thesis and forms the statistical basis for the risk estimation of the different risk factors for radiographic progression of joint damage.

In **Part II** of this thesis, we studied in more detail the relationship between serum markers and both the chances for a patient to develop RA and a subsequent worse outcome of RA. The markers we studied in this thesis all reflect a certain part in the chain of inflammatory processes that take place in the rheumatic joint as described in the introduction.

In the last decade, next to the older RF test, a new test against a collection of citrullinated proteins was developed, e.g. anti-CCP1, and improved to a second generation test that is widely used nowadays, anti-CCP2. The last few years also the third generation anti-CCP(3) test was developed as well as a test specifically directed against modified citrullinated vimentin (anti-MCV), for which performances similar to anti-CCP2 were reported.[71,72] Thus far, the performance for these autoantibodies was not subjected to a head-to-head comparison and the additive effect for each of these autoantibodies has not been subjected to a thorough investigation. In **chapter 3**, we compared all four autoantibodies, RF, anti-CCP2, anti-CCP3 and anti-MCV, for their usefulness in predicting RA development and looked at the predictive abilities for the rate of joint damage and the achievement of DMARD-free remission. Also the cumulative effect of performing multiple autoantibody tests was studied.

With the development of the revised 2010 criteria not only the aspect of presence or absence of ACPA was given weight in the prediction of RA development, but also higher autoantibody levels of RF and/or ACPA were valued more predictive in the classification of RA during the process of its development.[11,73] Although it has been shown that higher levels of RF and ACPA autoantibodies show a higher specificity than lower levels respectively, so does ACPA-positivity.[74-76] The question therefore arises, what is the value of incorporating RF-levels in the new criteria compared to ACPA-positivity, especially since in the new criteria RF and ACPA are regarded as equally predictive.[11] In an effort to improve the new criteria, in **chapter 4** we studied the value of higher levels of RF, defined as a cut-off level of 50 U/ml as used in literature,[74,75] and three times the cut-off for antibody positivity (3xULN) according to the definition in the new criteria,[11] and compared the results for multiple outcome measures with those obtained for ACPA-positivity.

Important factors in the inflammatory processes that take place in RA, as indicated in the introduction, are cytokines. In **chapter 5** the association of serum levels of one of these cytokines, CXCL13, and erosiveness was studied. CXCL13, also known as B lymphocyte chemo-attractant

(BLC) or B cell-attracting chemokine 1 (BCA-1), selectively attracts B lymphocytes,[77] and may play an important role in the process of bone remodeling through the interaction with its receptor CXCR5 that is also found on human osteoblasts.[78-81] The initial association between this serum marker and the amount of bone loss was predicted using an artificial computer model that mimics the processes in the joint of an RA-patient.

The last few years, genetic polymorphisms have been a focus of attention for establishing risk profiles in RA. New genetic regions have been identified to play a role in -primarily- the development of RA. Especially the use of whole genome scans has resulted in these multiple new targets. In **part III**, we report our findings for a selection of these factors.

Recently, several new polymorphisms were identified in a genetic region that is close to tumor necrosis factor α-induced protein 3 (*TNFAIP3*), involved in regulating TNF-receptor-mediated signaling effects.[82] In the ACPA-positive subgroup of patients, these polymorphisms have been observed to associate with a higher susceptibility for RA.[36] Hypothetically, these polymorphisms could associate with the severity of RA as well, but thus far this question has not been answered. In **chapter 6**, we studied if these polymorphisms, initially identified as risk factors for RA susceptibility, show an association with radiographic progression of joint damage in RA patients as well.

Similarly, in **chapter 7**, 6 SNPs that were recently identified in a genome wide association study to associate with the development of RA, were studied for an association with the outcome of RA. These polymorphisms are located in several genes, e.g. *CD40, KIF5A-PIP4KC, CDK6, CCL21, PRKCQ* and *MMEL1-TNFRSF14*, that have functions that are not only restricted to the immune systems response, but also are involved in regulation of the cell cycle progression.[37]

The third polymorphism studied for a possible association with the rate of joint destruction is located in the region of the protein tyrosine phosphatase non-receptor 22 (*PTPN22*). This region encodes a negative regulator of T-cell activation, and has been observed to be a risk factor for RA susceptibility in the ACPA-positive subgroup of patients.[83] Although this polymorphism was studied for an association with the outcome of RA several times, no consistent results were observed, possibly due to differences in methods of measurement and analysis. In **chapter 8**, we tried to clarify the role of *PTPN22* in the disease outcome of RA, using sensitive methods for scoring and analysis in two large cohorts of RA patients, both in the total group of patients and in the ACPA-positive subgroup.

The classic image of RA has been marked as a slow developing disease, and as such would provide opportunities by identifying RA patients as soon as possible for initiating treatment promptly. Years of experience in the treatment of RA patients have led to the hypothetical existence of a "golden" three month period for treating patients, the so-called window of opportunity.[84] Although it is a common rationale that increased symptom duration, e.g. a delay in visiting a rheumatologist and subsequent treatment, leads to a worse outcome of RA, the exact properties in terms of outcome measures and patient characteristics have not been studied in great detail

thus far. In **chapter 9** of **part IV,** we study the effect of symptom duration on the rate of joint destruction and the achievement of DMARD-free remission. In addition, since both patients and general practitioners can contribute to the total duration of the delay, we quantified these delays and studied various characteristics, among which ACPA, if associating with these delays. In addition to the presence of ACPA also the number of ACPA isotypes has been observed to associate with radiographic joint damage.[85] Therefore, the effect of the autoantibody response was studied for an association with the delay in ACPA-positive RA patients as well (**chapter 10**).

In **part V** of this thesis, we will address different clinical subphenotypes of RA. To identify factors that can be useful in the prediction of a disease, analysis of subphenotypes and extreme phenotypes can be valuable in addition to "more standard" cohort analyses. Although the number of patients representing the groups of the sub- and extreme phenotypes in general results in a lower number of patients available for analysis, it has been shown that these types of studies in fact can be informative.[86,87]

Reciprocal to the accumulation of joint erosions, is the occurrence of repair of joint erosions. Although joint damage was previously thought to be permanent, in the last few years the general state of mind shifted towards acknowledging that repair does exist.[88] Clinically, next to early treatment of patients to prevent the occurrence of bone damage, achieving repair of damage once it has developed would be an additional attainable goal. To reach this goal, understanding the pathophysiological mechanisms underlying the processes of repair are of great interest. Although the processes involved in bone homeostasis are complicated, in **chapter 11** we studied the occurrence of repair in a first step of characterizing this phenomenon.

In RA, the typical course of the disease is characterized by the occurrence of inflammation and subsequent development of joint erosions. Although the classic dogma is that inflammation causes damage directly, progressing insights indicate that the relation between inflammation and joint damage might not be that straight forward and might have different causal pathways.[89] In **chapter 12**, we studied the relation between inflammation, measured by the cumulative amount of joint swelling over a period of 5 years, and the degree of erosiveness that accumulated over this same period. In concordance with the extreme phenotype approach, patients from the extreme groups of joint swelling and erosiveness were studied and their characteristics compared to achieve more insight in the association between clinical inflammation and subsequent damage to the bones of RA patients.

In **chapter 13** (**part VI**) of this thesis, the 1987 ACR and the 2010 ACR/EULAR criteria for RA were subjected to a head-to-head comparison. The older 1987 criteria have already been established and incorporated in clinical practice by rheumatologists. The recently revised 2010 ACR/EULAR criteria however, have thus far not been subjected to a thorough evaluation of its applicability in clinical rheumatologic practice. We studied the performance of the new 2010 criteria for predicting development of RA, as well as the use of MTX or any DMARDs during the

first year and disease persistency over 5 years and compared it to the performance of the 1987 criteria.

Subsequently, in **chapter 14**, a 2010 update of the achievements in prediction making using the Leiden EAC is given. In particular the discoveries from the last decade and their implications in explaining the variance, not only for development of RA from UA but also for the outcome of RA in terms of the long term progression in radiological damage have been studied, the latter thus far not reported in literature yet.

Finally, in **chapter 15**, all results of this thesis will be summarized and the implications for predicting the development and outcome of RA will be discussed.

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PART I

Methodology of analyzing RA severity data

CHAPTER 2

Comparison of methodology to analyze progression of joint destruction in rheumatoid arthritis

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ABSTRACT

Background

The field of genetics is reaching phenotypic disease aspects. Within rheumatoid arthritis (RA), progression of joint destruction is an important phenotypic feature. Genetic factors often have small effect sizes, making avoidance of phenotypic misclassification and discerning true effects from noise challenging. Assembling radiological measurements repeatedly in time harbors a smaller risk of misclassification than single measurements. Given serial measurements, different methods of analysis can be applied. This study evaluates different statistical methodology to analyze longitudinal data and its effect on the power of such a study.

Methods

Kruskal-Wallis, Linear Regression and Repeated Measurements Analysis (RMA) were studied, both cross-sectionally (testing for differences in joint destruction at individual time points) and longitudinally (testing for differences in progression rates). Of these tests, only RMA takes advantage of within-patient correlations in serial radiological measurements. Data of 602 early RA patients included in an inception cohort with yearly radiographs and 7-years follow-up were assessed. Genetic data of HLA-DRB1 Shared-Epitope alleles and rs675520 (*TNFAIP3-OLIG3*) were used as example.

Results

From all methods studied, cross-sectional and longitudinal RMA were most powerful. For example analyses using longitudinal RMA in the current data set yielded powers >95%, even in presence of missing radiographs. In particular in the presence of small effect sizes RMA was more powerful than linear regression. The preciseness increased with a higher number of available measurements per patient.

Conclusion

A repeated measurement analysis on subsequent radiographs provides the most powerful methodology to analyze longitudinal data.

INTRODUCTION

In medicine more than 600 genome wide association studies have been published; often revealing inconsistent findings.[1] Now the field of genetics is moving from qualitative traits (disease yes/no) to phenotypic disease aspects and disease outcomes, which are often quantitative traits. Correct determination of the phenotype is of most importance here. Within rheumatoid arthritis (RA), progression of joint destruction is a relevant outcome measure, reflecting the cumulative burden of inflammation over time. The severity of joint destruction is highly variable between patients. Thus far, little is known about the pathophysiology of this difference. In addition, several clinical and serological risk factors for a severe rate of joint destruction have been identified, but the variation explained by these factors is low (R^2 0.36).[2-4] Prediction models based on these variables could classify only ~50% of RA patients.[2,5,6] In order to increase the understanding of the mechanisms underlying joint destruction, additional risk factors need to be identified. Thus far, few identified genetic factors for joint destruction are replicated. The absence of replication can have several causes. Obviously, it may be due to false-positive results in the initial study. Secondly, the replication study could have been underpowered. It is challenging to obtain long-term radiological data of a large number of patients. Finally, differences between studies may occur when different radiological measures are studied or when different methods of analyses are applied. Since the effect sizes of genetic markers in complex diseases are often moderate to small, both sensitive measurements of joint destruction and powerful methods of analysis are necessary to prevent false negative findings.

It is discussed elsewhere that the use of a continuous method to measure the degree of joint damage is more sensitive and discriminative than usage of categorical measures such as the presence of erosions.[7] In addition it has been shown that serial measures in time per patient give a more accurate and precise estimation of the rate of joint destruction compared to single measurements. Therefore, whenever possible, RA patients are preferably studied prospectively and have radiographs made at subsequent time-points.[7] In the presence of serial quantitative measurements, different statistical methods for analysis are available and applied. The level of joint destruction can be compared between groups at individual time-points, with and without taking radiological data on other time-points into consideration. Alternatively, the progression over all time-points can be compared in one test. An additional challenge in analyzing longitudinal radiological data is how to deal with missing radiographs. Therefore, we aimed to compare currently used statistical methodology to analyze continuous data on joint destruction over time. The main outcome measure evaluated was the power. We therefore evaluated the power of analyses performed with different statistical methods on the same patients and genetic data. First the power of these methods was evaluated using data of genetic variants known to associate with joint destruction. Second, we compared the ability of the different methods to deal with missing radiological data, as well as the effect of the number of available radiographs on the power of the study.

PATIENTS AND METHODS

Patients

Radiological data were used of 602 RA patients (according to the 1987 ACR-criteria) that were included in the Leiden Early Arthritis Clinic cohort (EAC) in 1993-2006.[8] Median symptom duration at inclusion was 0.36 years. At baseline, the mean age was 56.1±15.8 years, 78% was female and 54% was ACPA-positive. Yearly follow-up data over 7-years was used. Radiographs of hands and feet were scored chronologically according to the Sharp-van der Heijde method (SHS) by an experienced reader.[9-11] 409 radiographs belonging to 60 randomly selected RA patients were rescored. The intraclass correlation coefficient was 0.91 for all scored radiographs, and 0.97 for the radiographic progression rate. Treatment strategies changed in time.[8,12] Patients included in 1993-1995 were initially treated with analgesics and subsequently with chloroquine or salazopyrin. From 1996-1998 chloroquine or salazopyrin was promptly started. From 1999-2006 patients were readily treated with methotrexate or salazopyrin. Twenty-eight of the 602 patients received anti-TNF treatment somewhere during the seven follow-up years. The frequency of anti-TNF users was equally distributed between periods of inclusion (3.3%, 4.7% and 4.7% respectively).

Methods to analyze joint destruction

The HLA-DRB1 Shared-Epitope (SE) alleles and rs675520 (*TNFAIP3-OLIG3)* are associated with joint destruction.[13-16] To compare different statistical methods, these two genetic variants were studied as example (Figure 1). Three statistical methods were studied, representing the major methods for analyses. Other not-applied methods are more or less similar to the methods applied here.

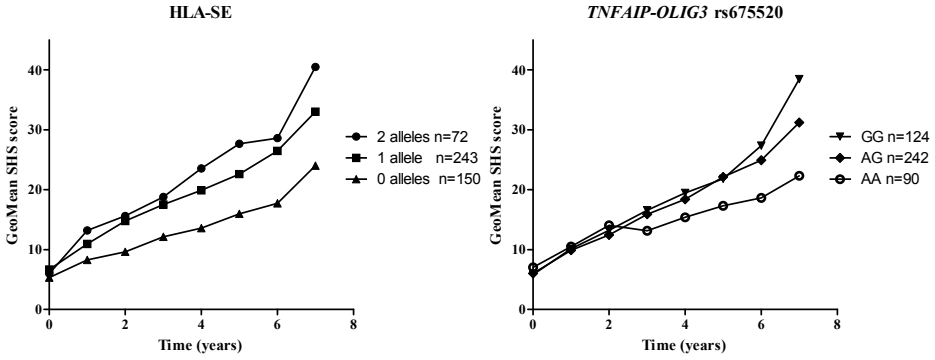

Figure 1. Sharp-van der Heijde scores during 7-years of follow-up for RA patients with 0, 1 or 2 HLA-SE alleles and with absence, presence or double presence of the minor allele of *TNFAIP-OLIG3* rs675520. Presented are the geometric means of the SHS

Cross-sectional methods studied, comparing destruction levels at individual time-points, were the Kruskal-Wallis test, linear regression analysis (LR_{cs}) and repeated measurement analysis (RMA_{cs}). The Kruskal-Wallis and LR_{cs} was performed on each time-point with SHS score as dependent variable ignoring the data of other time-points. For RMA_{cs}, a multivariate normal regression analysis was used with time as categorical variable.[17] The RMA_{cs} tested differences between SHS levels at each time-point taking radiological data on previous time-points into consideration.

The evaluated longitudinal methods, testing for differences in progression rates over time, were Kruskal-Wallis, longitudinal linear regression analysis (LR_{long}) and repeated measurements analysis (RMA_{long}). Here the Kruskal-Wallis test compared subtractions of SHS between baselines and the 7-years time-points and therefore data of only two measurements could be used. LR_{long} compared regression coefficients which are based on all available measurements, assuming them to be independent. RMA_{long} evaluated the progression rates over time considering the correlation between the measurements at all time-points within one subject. In order to have optimal comparisons of the tests, no adjustments were made in the LRs, and RMAs.

Since SHS were positively skewed, radiological scores were log-transformed to approximate normal distribution before performing any of the LRs and the RMAs. Analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL).[18]

Repeated measurement analysis

Detailed information on the used RMAs, a multivariate normal regression analysis, is provided in Box I, supplementary data. This analysis uses all available radiological measurements and has great flexibility to model time effects. It takes advantage of within patients' correlations and can handle missing data provided that the reason for missingness can be determined from the observed data (an assumption called missingness at random).[17,19]

The within-patient correlation of serial measurements is quantified by a covariance matrix. To determine the best-fitting covariance matrix the matrices available in SPSS were considered, using the Akaike information criteria as measure of goodness of fit. The heterogeneous first order autoregressive (ARH1) matrix was our final choice. It assumes a stronger correlation for measurements taken in a short period than taken over a longer period in time.

Power of different methods

It was hypothesized that the different methods will yield differences in power. To study this, the power to detect an association between the two genetic variants and joint destruction over 7-years was determined; both for the cross-sectional and longitudinal methods. For the Kruskal-Wallis, Quanto version 1.2.4[20] was used on the present data assuming that the effect of HLA-SE and rs675520 increased with respectively 1.3 and 1.2 times per year. The power of LR and RMA were computed by simulating the RMA model. The baseline characteristics of the patients, the sample size and parameter values were sampled such that they correspond to the original EAC

data. In order to also study the impact of missingness to the power, the percentage of missing radiographs was varied from 0 to almost 90% for the last visit. For the remaining visits, missingness was created with the same percentage as in the original dataset. More detailed description on the power analyses are described in the supplement. Power analyses were performed using R statistical software.[21]

Effect of number of radiological measurements

The number of measurements available per subject can differ between different study designs. Here we studied the influence of the number of measurements per subject on the preciseness of the estimation expressed as the 95% confidence interval (95%CI) of the effect size. To this end 107 patients with complete yearly follow-up over 7-years were studied. By simulation an increasing number of radiographs were left out between baseline and the 7-years time-point. In this way analyses were repeated with a lower number of radiological measurements per patient. Analyses were done on HLA-SE and joint destruction analyzed with both LR_{long} and RMA_{long}.

Missing radiological data in relation to different methods

The presence of missing data in longitudinal cohort studies is inevitable. Exclusion of the patients with missing data will generate bias in case missingness is related to the outcome of interest.[22] From the methods evaluated here, RMA is able to deal with missing data provided that the missingness is 'at random' or 'completely at random' and that the correlation structure (expressed by the covariance matrix) of the patients with missing data is comparable to that of patients with complete radiological data. Therefore the characteristics of missing radiological data in the studied cohort were evaluated.

RESULTS

Methods to analyze joint destruction

The cross-sectional and longitudinal methods of analysis were compared using radiological data of RA patients with different numbers of HLA-SE alleles. The various methods all resulted in significant outcomes at individual time-points (cross-sectional analyses) as well as on progression over time (longitudinal methods). The width of the 95%CI differed between the methods (see Table I).

Power and preciseness of different methods

The power to detect an association of HLA-SE with levels of joint destruction at the individual time-points from baseline till 7-years with Kruskal-Wallis in the present dataset were 0.52, 0.37, 0.40, 0.34, 0.36, 0.41, 0.48, 0.47. For rs675520, the power were 0.53, 0.31, 0.29, 0.22, 0.21, 0.19, 0.20, 0.18 from baseline till 7-years. Comparing differences in SHS between baseline and 7-years with Kruskal-Wallis had a power of 0.92 and 0.25 for HLA-SE and rs675520 respectively. The effect of missingness on the power of LR and RMA, both cross-sectional and longitudinal, are

illustrated by a simulation for different frequencies of missingness in Figure 2. The power to detect a difference in the cross-sectional analyses of HLA-SE groups was approximately 100% if the data at 7-years were complete. With increasing missingness the power of LR_{cs} diminished to <80%, whereas the power of RMA_{cs} remained >95%, even in case of a large percentage of missingness (Figure 2A). Although the power to detect a difference was lower in the analysis of rs675520, again it was observed that the power of RMA_{cs} remained higher than of LR_{cs}. Also for the longitudinal analyses, RMA had a higher power compared to LR (Figure 2B), for both HLA-SE and rs675520.

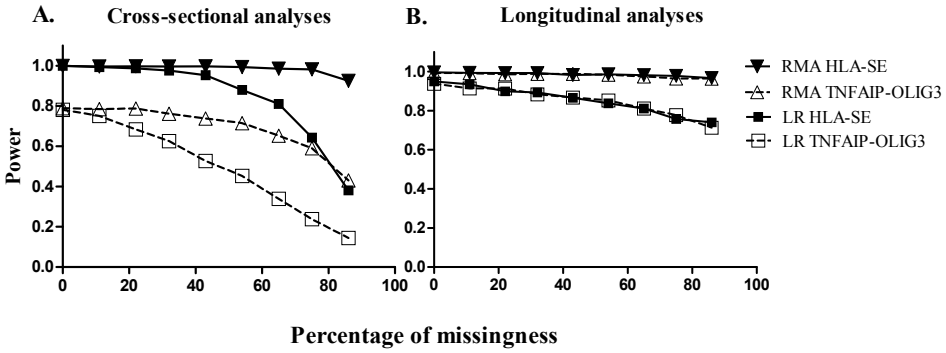

Figure 2. Power to detect differences in joint destruction with (A) cross-sectional and (B) longitudinal methods (LR and RMA) for different percentages of missing radiographs at the last time-point. Depicted is the power (y-axis) to detect an association between two different genetic variants, HLA-SE and *TNFAIP-OLIG3* (rs675520) and the rate of joint destruction in the present RA patients at the 7-years time-point.[24] The power was calculated (A) cross-sectional with linear regression (LR) and repeated measurement analysis (RMA) *at* 7-years and (B) longitudinally with LR and RMA *over* 7-years with different percentages of missing radiographs at the 7-year time-point (x-axis)

Effect of number of radiological measurements

With an increasing number of available radiographs the 95%CI of the estimation of the progression rate decreased, indicating a more precise estimation in the presence of more measurements per subject (see Figure 3).

Missing radiological data

Three major causes were identified that together accounted for >90% of all missing follow-up data: sustained DMARD-free remission (n=64), death (n=74), and not having complete follow-up data because of recent inclusion. Patients without sustained DMARD-free remission had a 2.35 (95%CI 1.83-3.19 p<0.001, RMA_{long}) times larger increase in SHS per 7-years. Patients had a constant 2.09 (95%CI 1.65-2.65 p<0.001, RMA_{long}) times larger joint damage over 7-years compared to those who stayed alive. For both reasons of missing data the missingness related to the outcome (missingness at random).

Table I: Risk estimates and p-values resulting from different statistical analyses associating the number of HLA-SE alleles with Sharp-van der Heijde scores during 7-years

		β (95% CI) *P-value*							
		Baseline	1	2	3	4	5	6	7
Cross-sectional analyses	Kruskal-Wallis#	N/A *0.244*	N/A *0.006*	N/A *0.002*	N/A *0.011*	N/A *0.007*	N/A *0.019*	N/A *0.037*	N/A *0.047*
	Linear regression † (LR_{cs})	1.10 (0.95-1.26) *0.20*	1.27 (1.1-1.48) *0.002*	1.32 (1.12-1.56) *0.001*	1.29 (1.08-1.54) *0.005*	1.34 (1.11-1.61) *0.003*	1.33 (1.10-1.63) *0.006*	1.29 (1.04-1.62) *0.02*	1.31 (1.01-1.66) *0.03*
	Repeated Measures Analysis (RMA_{cs}) †‡	N/A∞	1.25 (1.1-1.42) *0.004*	1.25 (1.11-1.40) *<0.001*	1.27 (1.15-1.42) *<0.001*	1.25 (1.14-1.37) *<0.001*	1.22 (1.13-1.32) *<0.001*	1.19 (1.12-1.25) *<0.001*	1.23 (1.07-1.41) *<0.001*
Longitudinal analyses	Kruskal-Wallis	N/A *0.008*							
	Linear regression* (LR_{long})	1.16 (0.94-1.43) *0.18*							
	Repeated measures Analysis* (RMA_{long})	1.25 (1.09-1.43) *0.002*							

The cross-sectional analyses compare the differences in SHS level at each time-point separately. The longitudinal analyses compare the differences in progression rates over time

\# This analysis does not result in a risk estimate.

† The β of the LR_{cs} and RMA_{cs} indicates the relative increase in SHS per risk allele at the individual time-points.

∞ Baseline was the reference in this analysis, therefore no risk estimate and p-value are present.

‡ The β of the RMA_{cs} indicates the relative increase in SHS per risk allele at the individual time-points.

* The β of the LR_{long} and RMA_{long} indicates the relative increase in SHS-progression per risk allele for the progression *over* 7-years.

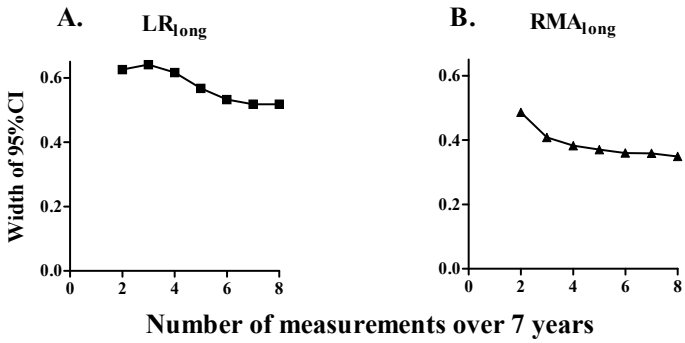

Figure 3. Width of 95% confidence interval (95%CI) for different number of measurement over 7-years of follow-up for (A) Linear regression analysis and (B) Repeated measurement analysis. Depicted is the 95%CI width (y-axis) of the analyses of the association between HLA-SE and joint destruction. The analysis was performed on 107 patients with complete follow-up yearly over 7-years. First only baseline and 7-years data was used, additional time-points were added to test the effect of the number of measurement used over the same time-period. A) The width of the 95%CI analyzing HLA-SE with LR_{long} demonstrates the advantage of adding more measurements to the analyses. B) The width of the 95%CI analyzing HLA-SE with RMA_{long} demonstrates the advantage of more measurements plus taking the correlation into account

DISCUSSION

The field of genetics is moving from disease susceptibility studies to studies addressing disease outcomes. Since genetic risk factors generally have small effect sizes, it is crucial to measure the outcome sensitively and to apply powerful statistical methodology. Given the presence of repeated radiologic measurements in time, different statistical tests can be used. We aimed to derive optimal statistical methodology. We considered commonly used methods but did not intend to give a complete overview of all possible statistical methods. We observed that, among the methods tested, a RMA is most powerful and least susceptible to bias. The increased power is the result of taking advantage of the high within-patient correlation in repeated measurements. We also observed that effect estimates were more precise in the presence of a higher number of measurements, an effect which is not specific for RMA. A RMA can compare absolute differences in SHS levels at a single time-point and rates of progression over time; the choice between these two may depend on whether one is interested in identifying associations with the level of joint destruction at a specific time-point or in identifying associations with the speed of progression of radiological joint damage over time.

We considered commonly used methods but did not intend to give a complete overview of all possible statistical methods. Advantages and disadvantages of the methods studied are presented in Table II. Advantageous of RMA is that all patients, also those who had missing radiographs, are included. This is done assuming that missing radiological scores can be estimated using available measurements and complete datasets of patients with similar characteristics, a situation called 'missingness at random'. Identified causes for missing radiographs in the present study

Table II: Advantages and disadvantages of the tested statistical methods to identify risk factors for joint destruction in RA

		Description	Can adjust for interfering factors	Includes serial measurements in one analysis	Includes patients with missing radiographs	Reliably deals with missing data	Uses within patient correlation (covariance matrix)
Cross-sectional analyses	Kruskal-Wallis	Tests the difference in rank of a continuous variable among the categories of a second variable	–	–	–	–	–
	Linear regression (LR_{cs})	Tests mean differences of continuous variable among the categories of a second variable	+	–	–	–	–
	Repeated Measures Analysis (RMA_{cs})	Idem previous	+	+	+	+	+
Longitudinal analyses	Kruskal-Wallis (progression between baseline & 7-year)	Tests the difference in rank of a continuous variable among the categories of a second variable	–	–/+	–	–	–
	Linear regression (LR_{long})	Tests differences of mean evolution of continuous variable among the categories of a second variable	+	+	+	–	–
	Repeated measures Analysis (RMA_{long})	Idem previous	+	+	+	+	+

– No, it does not

+ Yes, it does

were assumed to be missing at random, a requirement for adequate handling of missing data by the RMA. The RMA takes into account the uncertainty of the estimation for patients with missing radiographs. In other words, patients with complete datasets are weighted more heavily in the analysis than patients with missing radiographs. The RMA is the only studied method that did not exclude patient with missing data, which prevents certain bias.

Another, simple and frequently used method to deal with missing radiographs is a completers only analysis. Here all patients with missing observations are excluded. This is used when comparing differences in SHS between 7-years and baseline with Kruskal-Wallis tests and can lead to conflicting results at different time-points. An alternative is the last-observation-carried forward approach; this uses the last observation for every subsequent missing. Both methods can create bias since we observed that patients that are more inclined to have missing radiographs have relatively severe or relative mild joint destruction.[23]

The longitudinal LR studied compared the regression coefficients of SHS with time between groups. An advantage of LR above Kruskal-Wallis is that it gives an effect size and allows adjustment of correction variables. A drawback of LR is that it ignores the correlation between serial measurements; accounting for this would have resulted in a smaller standard error and therefore a more sensitive analysis. An alternative LR analysis over time is a two-step approach;[24] first a regression coefficient of SHS over time for each individual is estimated, which are then compared between groups. Although this method takes into account the correlation of the serial measurements within one subject, it ignores the standard error of these individual coefficients. Therefore, standard errors obtained with this approach are generally too small, introducing the risk of false-positive findings.

The RMA used in this manuscript is a multivariate normal regression analysis.[17] An alternative statistical method to analyze repetitive measurements is Generalized Estimating Equations (GEE),[25] which is occasionally used in clinical trials.[26,27] Advantages of GEE are that the data do not have to be normally distributed and the correlation structure does not have to fit the data. A disadvantage of GEE is that it assumes that missingness is 'completely at random', which is often not the case.[28] An extension of GEE, GEE with inverse probability weights,[29,30] can deal with missing data that is not completely at random, but this extension is not readily available in standard software packages. Since for GEE the correlation structure does not have to fit the data, GEE is often less precise than a multivariate normal regression. Since in the present cohort missingness was not 'completely at random', we preferred multivariate normal regression over GEE.

In the present study, no adjustments were made in the LRs and RMAs in order to increase the comparability of the tests. However, in studies evaluating associations with risk factors, it will be relevant to adjust for factors that interfere with or modify levels of joint damage, such as treatment. Adjustments generally result in a more precise estimation since the residual variance is decreased.

In conclusion, identification of new risk factors for RA severity is important. Genetic risk factors generally have moderate to small effect sizes. Therefore it is important to differentiate true

effects from noise and to have powerful methods of analysis. The present study demonstrated that a repeated measurement analysis on subsequent radiographs provides a sensitive method to analyze associations with joint destruction over time in longitudinal cohort studies.

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SUPPLEMENTARY DATA

Methods of power calculations

To show the power loss in detecting genetic effects when the within-patient correlation is ignored, we simulated data from the RMA (I) and (II) for the longitudinal and cross-sectional analyses, respectively. In the following section we will discuss the simulation setup in terms of the RMA (I).

First the RMA (I) is fitted to the EAC data in order to obtain estimates for the regression coefficients (namely intercept, $\beta_{\_j}$, γ and δ) and the variance components Σ. Then baseline characteristics for 602 patients are simulated based on the EAC patients' information. In particular, regarding sex 68% are women and 32% men and their age has been simulated from a normal distribution with mean 56 and standard deviation 16. In addition, the patients were assumed to have enrolled at different inclusion periods, i.e., 18% in the first, 35% in the second and 13% in the third. Regarding the genotypic information, genotypes have been simulated such that the minor allele frequency equals 0.41 and 0.37 (similar to the HLA-SE and *TNFAIP-OLIG3* in the EAC study). Finally, 8 yearly measurements are assumed to have been scheduled for all the patients. Using the baseline characteristics longitudinal responses Y_{ij} are simulated under model (I). To induce missingness at the last visit we randomly deleted 0-85% of the recorded values. The simulation of the longitudinal responses (for each missingness percentage) has been repeated 2000 times. In each of the 2000 simulated datasets both the RMA model (I) and LR_{long} are fitted and for each model we counted the number of times (out of the 2000) that the null hypothesis $\delta = 0$ is rejected. Thereby we compute the power to detect a genetic effect with effect size equal to that estimated for the EAC patients for different missingness percentages at the last visit. The same procedure is followed when model (II) is considered.

BOX I: Formula of RMA's

General formula of multivariate normal regression:

$Y_{ij} = \text{intercept} + \beta_1 x_{ij1} + \ldots. + \beta_p x_{ijp} + \varepsilon_{ij}$, $\quad i = 1, \ldots, n, j = 1, \ldots, T$

Y_{ij}= outcome from patient *i* at time-point *j*.

β_p = coefficient of P

P = covariate / interfering variables

ε_i = error terms, we assumed a multivariate normal distribution with mean vector zero and variance-covariance matrix Σ.

Here, the outcome is written as a linear function of a set of P covariates x_{ijp}

So the RMA_{long} concerns the following formula:

$Y_{ij} = \text{intercept} + \beta_{\_j} * [time_{ij}=t_j] + \gamma * \text{risk factor}_i + \delta * time_{ij} * \text{risk factor}_i + \varepsilon_{ij}$, (I)I = 1, …, 602, j = 1, …, 8

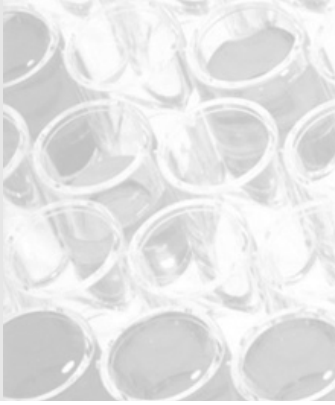
γ = the main group effect not changing over time

δ = the difference in increase of the outcome per year.

$time_{ij}=t_j$, time as factor, this allows the mean increase in response to diminish over time.[20,21]

For the RMA_{cs} the risk factor was entered with an interaction of time as categorical variable:

$Y_{ij} = \text{intercept} + \beta_{\_j} * [time_{ij}=t_j] + \gamma * \text{risk factor}_i + \delta_j * [time_{ij}=t_j] * \text{risk factor}_i + \varepsilon_{ij}$, (II)i = 1, …, 602, j = 1, …, 8



PART II

Serology in risk prediction of RA development and severity

CHAPTER 3

Value of anti-MCV and anti-CCP3 compared to anti-CCP2 and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis

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ABSTRACT

Objective

Autoantibodies such as rheumatoid factor (RF) and the second generation anti-cyclic-citrullinated-peptide autoantibodies (anti-CCP2) are frequently measured in clinical practice because of their association with disease outcome in undifferentiated arthritis (UA) and rheumatoid arthritis (RA). Recently two new tests were developed: anti-CCP3 and anti-modified-citrullinated-vimentin autoantibodies (anti-MCV). To facilitate the decision of which autoantibody to test in daily practice, this study evaluates aforementioned autoantibodies and combinations of them for predicting three outcome measures: progression from UA to RA, the rate of joint destruction and achieving sustained DMARD-free remission in RA.

Methods

625 UA patients were studied for the progression to RA after 1 year. 687 RA patients were studied for achieving sustained DMARD-free remission and the rate of joint destruction during a median follow-up of 5 years. Positive predictive values (PPVs) for RA development and the associations with the disease course in RA were compared for single tests (anti-CCP2, anti-CCP3, anti-MCV, RF) and for combinations.

Results

Using a single test in UA patients revealed that anti-CCP2 tended to have the highest PPV for RA development (67.1%), but the 95% confidence intervals of the other tests overlapped. Using a single test in RA, all tests showed comparable associations with the rate of joint destruction and achievement of remission. In ACPA-positive and ACPA-negative RA, RF-presence did not associate with more joint destruction. For all outcome measures, combining two autoantibody tests did not increase the predictive accuracy compared to performing one test.

Conclusion

For clinical practice, a single autoantibody test is sufficient for risk estimation in UA and RA.

INTRODUCTION

Rheumatoid arthritis (RA) is considered to have an autoimmune origin because of the presence of self-reactive autoantibodies. In addition to rheumatoid factor (RF), to date the only serologic measure included in the 1987 American College of Rheumatology criteria for RA, in recent years several other autoantibodies have been described.[1] The discovery of anti-citrullinated protein autoantibodies (ACPA) has led to the development of various new tests for autoantibodies in RA. The first generation anti-cyclic-citrullinated-peptide (anti-CCP) test, directed against a synthetic citrullinated peptide, revealed a higher specificity than RF (91-96% vs. 74-91%).[2-6] Subsequently, a commercially available second generation anti-CCP test (anti-CCP2) was developed, showing an even better specificity (90-97%).[3-8] RF and anti-CCP2 autoantibodies can also be present in the preclinical phase and are associated with future RA development.[9,10] Consequently, tests for anti-CCP2 and RF are nowadays widespread used as diagnostic tools in clinical practice.

Recently two other serological tests emerged, anti-CCP3 and anti-MCV. The anti-CCP3 test has been reported to have sensitivities and specificities comparable to anti-CCP2 (69-83% and 93-95% respectively).[8,11] The second novel autoantibody test targets modified citrullinated vimentin (MCV). This test has its origin in the older anti-Sa autoantibody test that has been shown to target citrullinated vimentin.[12] Compared to anti-CCP2, studies reported somewhat lower specificities and higher sensitivities for anti-MCV (79-92% and 70-84%).[3-5,13]

The aforementioned data were obtained by case-control studies comparing RA patients with non-RA patients or healthy individuals and the resulting test characteristics quantify the proportion of patients that are identified as positive by the test (sensitivity) or the proportion of healthy individuals that are identified as negative (specificity). As such, these measures, as well as the likelihood ratio of a test, provide information on the quality of the test. In clinical practice, the value of determining ACPA or RF relates to their ability to predict the disease course. The chance for an individual patient to have a certain disease course is expressed by the positive predictive value (PPV) and negative predictive value (NPV). A clinical state in which knowledge on the presence of RF and ACPA can be particularly helpful is undifferentiated arthritis (UA). In this subgroup of early arthritis patients no diagnosis can be established according to existing classification criteria and the presence of RF or anti-CCP2 indicates an increased risk for RA development.[14,15] Thus far the PPV and NPV for the risk to develop RA in UA have not been studied for anti-CCP3 and anti-MCV and the four autoantibodies have not been subjected to a head-to-head comparison. Furthermore, the additive value of testing several combinations of autoantibodies for the prediction of RA development in individual UA patients has not been addressed. Therefore, the first aim of this study is to compare anti-CCP2, anti-CCP3, anti-MCV and RF in the prediction of the RA development in patients with UA and to explore whether testing combinations of autoantibodies increases the predictive accuracy.

RF and anti-CCP2 are not only important predictors for RA development, but are also some of the most potent predictors for the outcome of RA, as measured by the rate of radiological

joint destruction.[16-18] Thus far, only one study compared radiological progression for anti-MCV and anti-CCP2 in 273 RA patients and provided suggestive evidence that anti-MCV is a better predictor than anti-CCP2.[19] The effect of testing combinations of all four autoantibodies however was not studied. Thus, the second aim of the present study is to compare anti-CCP2, anti-CCP3, anti-MCV and RF in the prediction of the rate of joint destruction and to explore whether combinations of autoantibodies can increase the predictive ability, taking advantage of a longitudinal cohort of 687 RA patients with a median follow-up of 5 years.

A second disease outcome of RA is the achievement of remission that with the introduction of new aggressive treatment modalities has increasingly become an attainable goal. We chose a strict definition and defined sustained disease-modifying antirheumatic drugs (DMARD)-free remission as the persistent absence of synovitis for at least 1 year after cessation of DMARD-therapy.[20] Since the predictive value of the four autoantibodies in relation to remission is scarcely explored, the present study compares the four tests for their ability to predict sustained DMARD-free remission in RA patients treated with conventional DMARDs.

In summary, to support the choice on which autoantibody to test in daily practice, this study uses a large longitudinal cohort to evaluate the value of determining anti-CCP2, anti-CCP3, anti-MCV and RF for predicting three outcome measures: progression from UA to RA, the rate of joint destruction and the chance of achieving sustained DMARD-free remission in RA. In addition, the predictive value of combining several tests is investigated.

PATIENTS AND METHODS

Patients

All patients included in this study are selected from the Leiden Early Arthritis Clinic (EAC) cohort that was started in 1993.[21] Patients were referred by general practitioners when arthritis was suspected. Inclusion took place when arthritis was confirmed at physical examination and symptom duration was less than 2 years. Written informed consent was obtained from all participants. The study was approved by the local Medical Ethical Committee.

At inclusion, patients were inquired about their joint symptoms and subjected to a physical examination. Blood samples were taken for routine diagnostic laboratory screening (including IgM-RF) and stored to determine other autoantibodies at a later time. Follow-up visits were performed on a yearly basis and included radiographs of hands and feet.

Since the start of the EAC treatment strategies for RA have changed; four different strategies were applied depending on the inclusion period. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or salazopyrin if they had persistent active disease (delayed treatment).[22] From 1996 to 1998 RA patients were promptly treated with either chloroquine or salazopyrin (early treatment).[21,22] From 1998 to 2002 patients were promptly treated with either salazopyrin or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either salazopyrin or methotrexate com-

bined with treatment adjustments based on the disease activity (early and disease activity based treatment). Treatment of UA patients was not protocollized.

UA was defined as not fulfilling one of the existing classification criteria for rheumatologic diagnoses two weeks after the first presentation.[23] Thus other rheumatic diseases like Sjögren's syndrom, psoriatic arthritis, spondylarthropathies, etc. that were established at baseline were excluded (Figure 1). 625 patients with UA, consecutively included between 1993 and 2006, were studied. After 1 year of follow-up, 201 patients (32.2%) had progressed to RA, fulfilling the 1987 ACR criteria for RA. In addition, 687 patients with RA included between 1993 and 2006 were studied for their disease outcome; 486 patients fulfilled the ACR criteria for RA already at inclusion and 201 patients were initially diagnosed with UA and developed RA within the first year of follow-up (Figure 1).

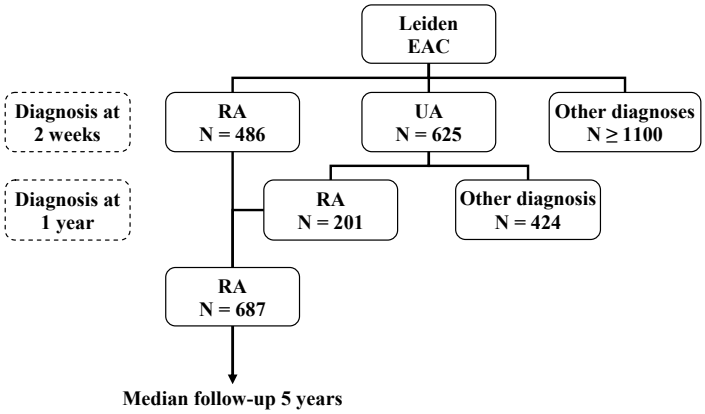

Figure 1. Flowchart of the study. From the 687 RA patients, 579 had radiographic data available and for 635 patients information on sustained DMARD-free remission was obtained. From all UA patients RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 623, 624, 597 and 597 patients respectively. From all RA patients with radiographs available RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 572, 565, 544 and 544 patients respectively. From all RA patients with data on sustained DMARD-free remission, RF, anti-CCP2, anti-CCP3 and anti-MCV were determined in 615, 603, 579 and 579 patients respectively

Autoantibodies

IgM–RF was determined by enzyme-linked immunosorbent assay (ELISA). Anti-CCP2 autoantibodies (total IgG) were measured by ELISA (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff level for anti-CCP2 autoantibody-positivity was set at 25 arbitrary units, according to the manufacturer's instructions. Anti-CCP3 autoantibodies (IgA and IgG subforms) and anti-MCV autoantibodies were measured with ELISA as well (Quanta lite CCP 3.1 IgG/IgA, INOVA Diagnostics Inc., San Diego, CA, USA and Orgentec Diagnostika GmbH, Mainz, Germany respectively). According to the manuals, the cutoff level for both tests was 20 arbitrary units. The numbers of patients in which RF, anti-CCP2, anti-CCP3 and anti-MCV measurements were performed show some little variation (available data for RF, anti-CCP2,

anti-CCP3 and anti-MCV were respectively 665, 653, 629 and 629 out of 687 RA patients and 623, 624, 597 and 597 out of 625 UA patients). This is due to the difference in timing of performing the tests. RF was determined routinely at inclusion whereas the ACPA were determined using stored serum samples. The recently introduced anti-CCP3 and anti-MCV assays were performed on all available sera in 2008, whereas the anti-CCP2 assay was performed earlier.

Radiographs

Radiographs of hands and feet were taken on consecutive years starting at baseline and were scored according to the Sharp-van der Heijde method.[24] From all RA patients, 579 had data on both autoantibodies and radiographs. To encompass a reliable sample size during follow-up, radiographic data were restricted to a maximum of 7 years of follow-up. The number of available radiographs varied per time-point and declined from 552 at baseline to 478 after 1 year, 426, 358, 299, 270, 207 and 156 after 2 till 7 years of follow-up respectively. Due to the study design (an inception cohort) not all patients had a similar duration of follow-up (median 5 years). All radiographs were scored by one experienced scorer (ML) who was blinded with respect to the patient's autoantibody status, treatment and other clinical data. Scoring was performed with known time order, which is more sensitive to change compared to scoring with unknown time sequence.[25] From the total number of scored radiographs, 499 radiographs were rescored by the same reader, consisting of 149 baseline radiographs and 350 radiographs during follow-up belonging to 60 randomly selected RA patients. The intraobserver intraclass correlation coefficients were 0.91 for all scored radiographs, 0.84 for baseline radiographs and 0.97 for the radiographic progression rate.

Sustained DMARD-free remission in RA

Remission was defined in its most stringent form as the persistent absence of synovitis for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheumatologist.[26] The remission status could be reliably ascertained in 635 RA patients. Most patients who achieved remission were followed-up longer than the minimum requirement of 1 year; the median time of observation after discontinuation of DMARDs in the absence of swollen joints was 2.5 years. Patients, who had a recurrence of their arthritis after discharge, could easily return to the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of approximately 400.000 inhabitants. The frequency of relapse was recorded and patients with relapse were included in the non-remission group (n=6).

Statistical analysis

The PPV (proportion of UA patients with a positive test that progressed to RA) and the NPV (proportion of UA patients that did not develop RA) were determined.

As radiographic data are not normally distributed, non-parametric Mann-Whitney tests were used to compare the Sharp-van der Heijde scores at individual time points for patients with and

without autoantibodies or for autoantibody combinations. In addition, to take advantage of the prospective character of the data consisting of repeated measurements, and to avoid multiple testing by performing statistical tests for each time point, a linear mixed model with an autoregressive correlation structure with heterogeneous variances was used. This model estimates the linear progression rate in radiological joint destruction using normalized, log-transformed Sharp-van der Heijde scores, taking missing observations into account. This means that it compares the progression rates for the different patient groups. In the mixed model analyses, corrections were applied for age, gender and inclusion period/treatment strategy. Correction for treatment strategy was performed by including the inclusion period in the linear mixed model. This was done because treatment modalities improved over time and an influence of the treatment strategy (reflected by the inclusion period) on the progression of radiographic joint damage was observed previously as well as in the present study (data not shown). In addition, the available follow-up duration differed between patients and therefore the number of radiographs per time point declined during follow-up. As the patients with the longest follow-up were included in the earliest inclusion period and thus have been treated with the least aggressive treatment strategy, correction for inclusion period was performed. In order to prevent overfitting of the data no corrections were applied for other variables. Analysis of sustained DMARD-free remission was performed by Cox regression analysis, to take into account the differences in follow-up times among patients. For patients that achieved remission the dependent variable is the "time-to-event", indicating the time until reaching remission. For non-remission patients the time to last follow-up was used, with a maximum of 10 years. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used. P-values <0.05 were considered significant. All reported p-values are two-sided.

RESULTS

Progression from UA to RA

From the total EAC, consisting of >2,000 patients with early arthritis, UA patients (n=625) were selected and studied for progression to RA after 1 year of follow-up. At inclusion, the mean age was 50.9 (±17.0) years, 371 (59.1%) patients were female and the self-reported symptom duration was 5.5 (±8.5) months. Anti-CCP2, anti-CCP3, anti-MCV and RF were present in 149 (23.9%), 172 (28.7%), 199 (31.7%) and 155 (24.8%) patients respectively. The presence of autoantibodies overlapped; UA patients who tested positive for anti-CCP2 were also frequently positive for other autoantibodies (Figure 2).

The PPV was compared for the four autoantibodies (Table 1). Anti-CCP2 had the highest PPV, 67.1%, compared to 64.0%, 56.3% and 61.7% for anti-CCP3, anti-MCV and RF respectively. The NPVs of all four tests were comparable (~80%). Thus, in case one autoantibody test is performed, a positive anti-CCP2 test tends to correlate with the highest risk of RA development, but overlapping 95%CI's hamper a definite differentiation.

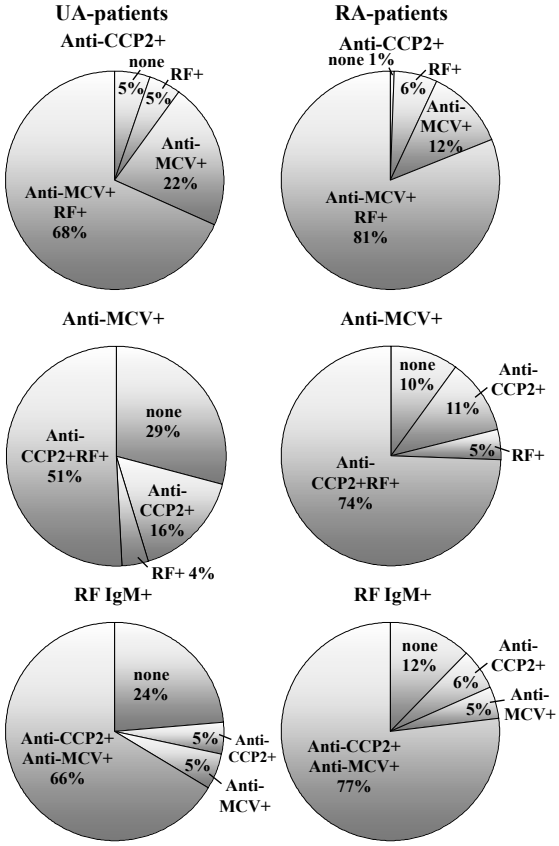

Figure 2. Prevalence of other autoantibodies in case of a positive test result for anti-CCP2, anti-MCV and RF for both UA and RA. Figure 2 illustrates the prevalence of other autoantibodies given one positive autoantibody test. This figure is derived using 596 UA and 540 RA patients for whom data on all autoantibodies was present. none: no other autoantibodies present

Next it was determined whether performing two autoantibody tests results in a better estimation of the risk for RA than performing one test. Since anti-CCP2 and anti-CCP3 are related tests and anti-CCP2 positive patients were in 90% also positive for anti-CCP3, the possible combinations of anti-MCV, anti-CCP2 and RF were first assessed. The proportions of patients that developed RA in case of two positive test results (PPV) were: anti-CCP2+/anti-MCV+ 69.9% (95%CI 62.1-77.7), anti-CCP2+/RF+ 74.1% (95%CI 65.8-82.3) and anti-MCV+/RF+ 70.6% (95%CI 62.1-79.2). Additional analyses using anti-CCP3 instead of anti-CCP2 yielded comparable results (data not shown). Altogether, these data show that when two tests are performed none of these combinations is clearly superior to the other. Furthermore, no additive value of performing two instead of one autoantibody test could be observed.

When performing a single autoantibody test, no information about the presence or absence of the other autoantibodies is obtained. However, the eventual coexisting presence of other

Table 1. PPV, NPV, sensitivity, specificity and likelihood ratios of anti-CCP2, anti-CCP3, anti-MCV and RF for predicting progression from UA to RA

Autoantibody Test	PPV		NPV		Sensitivity		Specificity		Likelihood Ratio	
	% (95%CI)	True pos./ all pos. tests	% (95%CI)	True neg./ all neg. tests	% (95%CI)	True pos./ all RA	% (95%CI)	True neg./ all non-RA	LR+	LR-
Anti-CCP2	67.1 (59.6-74.7)	100/149	79.0 (75.3-82.6)	375/475	50.0 (43.1-56.9)	100/200	88.4 (85.4-91.5)	375/424	4.33	0.57
Anti-CCP3	64.0 (56.8-73.1)	110/172	80.0 (76.2-83.8)	340/425	56.4 (49.5-63.4)	110/195	84.6 (81.1-88.1)	340/402	3.66	0.52
Anti-MCV	56.3 (49.4-63.2)	112/199	79.2 (75.2-83.1)	315/398	57.4 (50.5-64.4)	112/195	78.4 (74.3-82.4)	315/402	2.66	0.54
RF	61.7 (54.0-69.4)	95/154	77.8 (74.1-81.6)	365/469	47.7 (40.8-54.7)	95/199	86.1 (82.8-89.4)	365/424	3.43	0.61

UA: undifferentiated arthritis; PPV: positive predictive value; NPV: negative predictive value; 95%CI: 95% Confidence Interval; pos.: positive; neg.: negative; LR: likelihood ratio

unmeasured autoantibodies can affect the risk for RA. To determine the risk for RA as conferred to by the individual autoantibodies or by the number of autoantibodies, the PPVs for progression to RA were determined in the group of 596 UA patients for whom information on all three autoantibodies was available. The difference with above mentioned data is that the presence of two autoantibodies now indicates that the third is absent, whereas in above mentioned data only two tests were performed and the third autoantibody test could be positive as well as negative. The PPV for RA development in patients without autoantibodies was 18.8% (95%CI 14.7-22.9). In the presence of one autoantibody the PPV was 26.5% (95%CI 17.9-35.0) and increased significantly to 59.6% (95%CI 45.5-73.6) in the presence of two autoantibodies (Table I, supplementary data). The PPV was the highest in the presence of 3 autoantibodies (73.3%, 95%CI 64.6-81.9).

In conclusion, when performing one autoantibody test in clinical practice, none of the four tests is clearly superior. Although the presence of 2 autoantibodies significantly increased the risk for RA compared to the presence of 1 autoantibody, for clinical use performing two tests does not significantly increase the predictive performance compared to performing one test. This finding is likely explained by the presence of other, non-measured autoantibodies that affect the risk for RA.

Joint destruction in RA

Baseline characteristics of the 579 studied RA patients were: mean age 56.2 (±15.5) years, 405 patients (69.9%) were female, anti-CCP2, anti-CCP3, anti-MCV and RF were present in 313 (55.4%), 322 (59.2%), 331 (60.8%) and 334 (58.4%) patients respectively. Anti-CCP2 positive patients also tested positive for anti-MCV and RF in 93% and 87% of cases respectively (Figure 2).

First, the association between autoantibody-positivity and rate of joint destruction was assessed when performing only one autoantibody test. For all autoantibody tests (anti-CCP2, anti-CCP3, anti-MCV and RF) a positive test result was associated with a higher Sharp-van der Heijde score at all time points except baseline (M-W, $p<0.001$) and a higher rate of joint destruction over a period of 7 years (mixed model, $p<0.001$) compared to a negative test result. Figure 3A shows that there is no difference among the four tests with regard to predictive ability for joint destruction.

Next, it was investigated whether the addition of a second autoantibody test increased the predictive value for the rate of joint destruction. As depicted in Figure 3B no differences were seen between testing positive for anti-CCP2, anti-MCV or RF alone and combinations of these autoantibodies. These results indicate that for clinical use, a positive test for one of these autoantibodies predicts a severe disease course, and a second or third autoantibody test does not increase the predictive accuracy. Testing for anti-CCP3 instead of anti-CCP2 gave comparable results (data not shown).

To identify the contribution of the individual autoantibodies to the association with the rate of joint destruction, the effect of the number of positive autoantibodies (with the known absence of the other autoantibodies) was investigated using data on RF, anti-CCP2 and anti-MCV. The

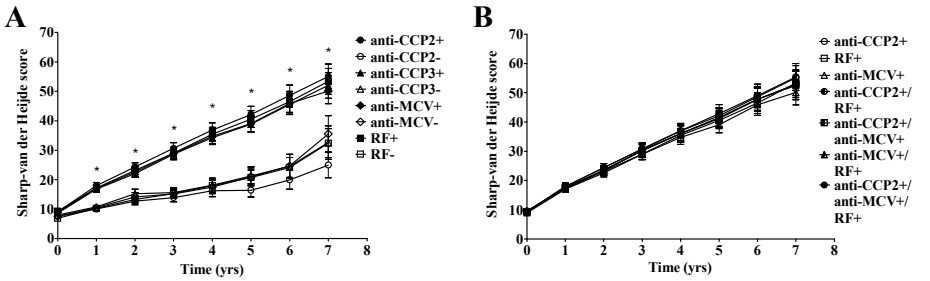

Figure 3. Mean Sharp-van der Heijde scores (±SEM) for the different autoantibody tests (A) and for combinations of positive tests (B). The number of patients in Figure 3A for anti-CCP2-positive/negative, anti-CCP3-positive/negative, anti-MCV-positive/negative and RF-positive/negative were respectively 313/252, 322/222, 331/213 and 334/238. *Mann-Whitney, p<0.001, for comparison of positive with negative test. For all four tests the p-value was <0.001 using mixed models. In Figure 3B, for anti-CCP2+, RF+, anti-MCV+, anti-CCP2+/RF+, anti-CCP2+/anti-MCV+, anti-MCV+/RF+ and anti-CCP2+/anti-MCV+/RF+ the numbers of patients were 313, 334, 331, 271, 283, 259 and 244 respectively

presence of either 2 or 3 autoantibodies was associated with a higher rate of joint destruction compared to 0 or 1 autoantibody (Figure 4A) (mixed model, p <0.001 for both 2 and 3 compared to 0 as well as 1 autoantibody). No significant difference was observed between the presence of 2 or 3 autoantibodies or between 0 and 1 autoantibody. It should be noted that the group with 1 autoantibody consisted almost exclusively of patients that were anti-MCV+ (n=33) or RF+ (n=39); only two patients were positive for anti-CCP2 and negative for anti-MCV and RF.

To more specifically investigate the role of RF in relation to ACPA (anti-CCP2, anti-CCP3 or anti-MCV) and the rate of joint destruction, the additional effect of RF in the presence or

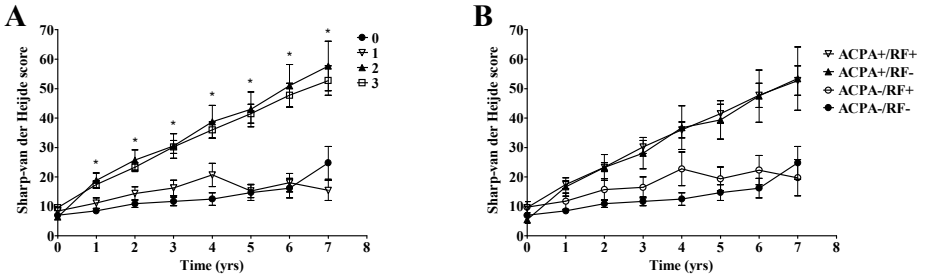

Figure 4. Mean Sharp-van der Heijde scores (±SEM) for the number of positive autoantibody tests (A) and for the effect of RF in the presence or absence of ACPA (B). In Figure 4A the mean Sharp-van der Heijde scores are depicted for the number of positive autoantibody tests (with the other tests negative) studying data on RF, anti-CCP2 and anti-MCV (total n=540). The numbers of patients positive for 0, 1, 2 or 3 autoantibodies were 152, 74, 70 and 244 respectively. Comparing 3 with 0, 3 with 1, 2 with 1 and 2 with 0 autoantibodies revealed a p<0.05 at all time points except baseline (M-W) and a p<0.001 for the 7 year period (mixed model). In Figure 4B the mean Sharp-van der Heijde scores are depicted for the following groups ACPA-RF-, ACPA-RF+, ACPA+RF- and ACPA+RF+, the patients numbers per group were 152, 39, 36 and 244. P=0.864 for comparison of ACPA-RF- with ACPA-RF+ and p=0.702 for comparison ACPA+RF- and ACPA+RF+ (both mixed model)

absence of ACPA was determined (Figure 4B). This revealed that in the presence of ACPA, but also in the absence of ACPA, RF did not significantly contribute to the rate of joint destruction.

Sustained DMARD-free remission in RA

From a total of 635 RA patients, 78 patients achieved sustained DMARD-free remission after a median follow-up of 39.5 months. These 78 patients had a mean age of 59.4 (±15.7) years, 57 (73.1%) were female. Anti-CCP2 autoantibodies were present in 11.8% and anti-CCP3, anti-MCV autoantibodies and RF in 21.9%, 28.8% and 25.0%.

The four autoantibody tests were compared for their association with the achievement of remission (Figure 5A). The Hazard Ratio (HR) of each of the four tests for not achieving sustained DMARD-free remission was 11.6 (95%CI 5.8-23.4) for anti-CCP2, 6.0 (95%CI 3.4-10.4) for anti-CCP3, 4.9 (95%CI 3.0-8.2) for anti-MCV and 4.7 (95%CI 2.8-8.0) for RF.

Subsequently, the additive value of performing two autoantibody tests compared to one test was investigated. The HRs were 15.6 (95%CI 6.7-36.4), 14.0 (95%CI 6.4-31.0) and 11.5 (95%CI 5.4-24.5) for combinations of anti-CCP2 and RF, anti-CCP2 and anti-MCV and anti-MCV and RF respectively. These data indicate that to predict the chance on remission, performing two tests has no additional value compared to anti-CCP2 alone.

To investigate whether the number of present autoantibodies affected the chance of achieving sustained DMARD-free remission, the HRs were determined for the presence of 1, 2 or 3 positive autoantibodies (with the other autoantibodies known to be absent) using data on anti-CCP2, anti-MCV and RF (Figure 5B). The HRs for not achieving sustained DMARD-free remission were as follows, 3.7 (95%CI 1.1-12.3), 15.5 (95%CI 5.9-41.2) and 17.1 (95%CI 6.8-43.3) for the presence of, respectively, 1, 2, and 3 autoantibodies compared to no autoantibodies. Thus, however the 95%CIs were overlapping, the results suggestively indicate that the more autoantibodies are present, the lower the chance is to achieve sustained DMARD-free mission.

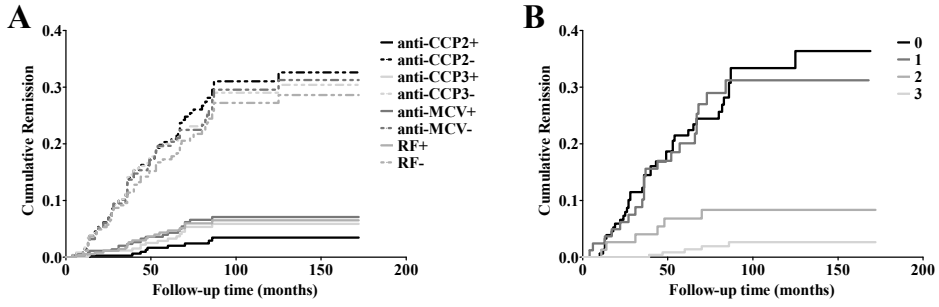

Figure 5. Effect of the presence of a positive autoantibody test (A) and the number of autoantibodies on sustained DMARD-free remission (B)

DISCUSSION

Among the autoantibodies tested in RA, only RF and ACPA are considered clinically useful. In clinical practice a physician or patient is interested in the chance for the individual to progress to RA or not, given a positive or negative test result respectively. These risks are reflected by the PPV and NPV. In addition, in early arthritis the predictive value of these autoantibody tests is valued the most in patients in whom at presentation no definite diagnosis can be established (UA), as only one-third of these patients progresses to RA after one year.[14] Also in RA, the autoantibody tests form one of the most potent predictors to obtain an indication on the severity of the future disease course. As such, information on the results of the autoantibody tests can influence treatment decision in individual patients with UA and RA.[23] Nevertheless, it is thus far unknown which test, or which combination of tests, is most powerful in predicting the progression from UA to RA and the disease progression in RA and therefore the present study was undertaken.

To evaluate the prediction from UA to RA using a single test, the PPVs of the four tests were compared. This revealed that a positive anti-CCP2 test tended to have the highest predictive value (PPV 67% compared to 62% and 56% for RF and anti-MCV), but due to overlapping 95%CIs a definite differentiation could not be made. In addition, performing a second test did show a tendency for a higher chance on RA development in case both tests were positive (highest risk for RA in anti-CCP2+, RF+ patients, PPV 74%), but also here the 95%CIs overlapped. To formally conclude whether the PPVs of 67% and 74% are statistically significantly different or not, >1800 UA patients would be required (using a p-value of 5% and a power of 90%). Based on the present data it is concluded that addition of a second test does not result in an increased predictive accuracy. Notably, UA patients who tested positive for anti-CCP2 were also frequently positive for other autoantibodies (Figure 2). Thus the finding of comparable prognostic performances of one or two tests is likely due to co-existing presence of autoantibodies that are unmeasured with a single test but that do affect the risk-estimation for RA.

The development of RA was assessed after 1 year of follow-up. This time-point was chosen in order to have a similar duration of follow-up for all studied patients. However, this may have introduced misclassification as patients with UA may have progressed to RA after more than 1 year of follow-up. With all available follow-ups, 25 patients (4.4%) progressed to RA later than 1 year, indicating that the current PPVs may be marginally underestimated. Since this misclassification is present in the total group of UA patients this does not hamper a comparison of tests.

Two measures were studied for the severity of the disease course in RA: achieving sustained DMARD-free remission and the level of radiological joint destruction during a median follow-up of 5 years. Although Figure 3A and 5A may lead to the impression that anti-CCP2 positive patients have a higher rate of joint destruction and achieve sustained DMARD-free remission less frequently than patients positive for the other autoantibodies, these differences were not statistically significant. Similar to the data on RA development, performing a second test appeared not to result in a more accurate prediction for the disease outcome in RA. This observation,

which is in contrast to an earlier report,[27] can be explained by the presence of non-measured autoantibodies that are associated with a progressive course of RA.To obtain a more detailed comprehension of the current results, the contribution of the individual autoantibodies to disease progression (RA development, joint destruction and achievement of remission) was investigated. This showed that the presence of two autoantibodies indicated a significantly increased risk compared to the presence of one autoantibody. However, the group with one autoantibody present consisted mostly of anti-MCV or RF-positive patients. Patients that were positive only for anti-CCP2 were very rare. Therefore it cannot be excluded that the increased risk in the presence of two compared to one autoantibody is due to the effect of anti-CCP2 rather than to the effect of an additional autoantibody. Nevertheless, in general it was observed that a higher number of autoantibodies present resulted in a higher risk for RA or for progressive disease. This is in line with recent published data showing that a broader autoantibody response associated with disease progression.[28]

As the association of RF with the presence of RA is primarily explained by its interaction with ACPA,[29] we investigated whether we could observe a similar effect for the progression of joint destruction in RA. Intriguingly, the rate of joint destruction both in the ACPA-positive and ACPA-negative groups was not affected by the presence or absence of RF. This finding further supports the notion that RF does not, by itself, contribute to disease progression.

In order to get an impression of the effect of anti-MCV itself on the rate of joint destruction, RA patients that were positive only for anti-MCV (n=33) were studied. This revealed that the rate of joint destruction was comparable to that of the patients with no autoantibodies (data not shown), indicating that anti-MCV alone does not strongly affect the level of joint damage in RA.

In conclusion, our results indicate that for risk estimation of the disease course in clinical practice, performing a single autoantibody test is sufficient, both in UA and RA.

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Supplementary data, Table I. Positive predictive values of the number of positive antibodies for progression from UA to RA

Number of Positive Antibodies	PPV % (95% CI)	True pos./ all pos. tests	Antibody Combinations	PPV % (95% CI)	True pos./ all pos. tests
0	17.9 (13.9-22.0)	61/340	-	-	-
1	27.5 (18.8-36.1)	28/102	Anti-CCP2+/anti-MCV-/RF-	12.5 (-10.4-35.4)	1/8
			Anti-CCP2-/anti-MCV+/RF-	29.3 (17.6-41.0)	17/58
			Anti-CCP2-/anti-MCV-/RF+	27.8 (13.2-42.4)	10/36
2	61.2 (47.6-74.9)	30/49	Anti-CCP2+/anti-MCV+/RF-	58.1 (40.7-75.4)	18/31
			Anti-CCP2+/anti-MCV-/RF+	83.3 (53.5-113.2)	5/6
			Anti-CCP2-/anti-MCV+/RF+	58.3 (30.4-86.2)	7/12
3	71.9 (62.9-80.9)	69/96	-	-	-

These data are based on the patients with data available of all three antibodies (anti-CCP2, anti-MCV and RF, n=587). UA: undifferentiated arthritis; PPV: positive predictive value; 95%CI: 95 % Confidence Interval; pos.: positive

CHAPTER 4

Towards a data-driven evaluation of the 2010 ACR/EULAR criteria for rheumatoid arthritis: Is it sensible to look at levels of rheumatoid factor?

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ABSTRACT

Objective

Recently new classification criteria for Rheumatoid Arthritis (RA) have been devised by methodology that used first a quantitative approach (data from databases), then a qualitative approach (consensus-based on paper patients) and finally a common sense based approach (evaluation of the former phases). Now these criteria are being evaluated to assess characteristics of the individual items. This study analyzed characteristics of the item autoantibodies, in particular RF level.

Methods

Three separate cohorts with a total of 972 undifferentiated arthritis patients were studied for RA development (according to the 1987 ACR criteria) and arthritis persistency. Positive and negative predictive values (PPV, NPV) and likelihood ratios (LR) were compared between different levels of RF and the presence of ACPA. A similar comparison was made in 686 RA patients for the rate of joint destruction during 7 years of follow-up and achievement of sustained DMARD-free remission. The variation in RF levels obtained by different measurement methods in the same RF-positive sera was explored.

Results

Presence of ACPA had a better balance between LR+/LR- and PPV/NPV than high RF levels for RA development. The additive value of ACPA assessment after high level RF testing was higher than vice versa. High level RF was less strongly associated with RA severity than ACPA antibodies. The RF level obtained by different methods in the same patients' sera varied considerably.

Conclusion

Level determination of RF is subject to large variation; high level RF has limited additive prognostic value compared to ACPA positivity. Thus, omitting RF level and using RF presence, ACPA presence and ACPA level may improve the 2010 criteria for RA.

INTRODUCTION

Recently, the American College of Rheumatism (ACR) classification criteria for rheumatoid arthritis (RA) dating back from 1987,[1] have been subjected to a process of rejuvenation by a joint taskforce of both the ACR and the EULAR (European League Against Rheumatism). The aim of these criteria is to classify RA in an earlier disease stage compared to the 1987 ACR criteria and the development of these criteria is an important step forwards.

The development of the 2010 ACR/EULAR criteria comprised three phases. First, a data driven phase using data from 3115 patients from Europe and Canada. Next, a phase incorporating the expertise of 39 rheumatologists and finally a consensus phase by the same group.[2-4] It is foreseen that in the next years the criteria will be studied in cohorts with different ethnic backgrounds and dissimilar healthcare systems in which the pretest probability for RA in new patients visiting rheumatologists differs.

The 2010 criteria are the first that include anti-citrullinated peptide antibodies (ACPA), in addition to RF. Presence of these auto-antibodies can contribute substantially to the classification of RA for which ≥6 points are required; presence of ACPA or RF yields 2 points and high levels of ACPA or RF yields 3 points. In the data driven phase of the development of the criteria, using data of several early arthritis cohorts, ACPA and RF were recognized as a theme in a factor analysis. Then, ACPA and RF were summarized as 'serology'. Subsequently the importance of serology, independent of other variables, was determined using a multivariate regression analysis. It was observed that within the patients with a positive serology, patients with a level higher than median received a higher weight than patients with a level lower than median. After the expert-phase and consensus-phase a high level was redefined as ≥ three times the reference value.

The present study aimed to provide two main characteristics of the items serology, particularly the RF level criterion, in the 2010 ACR/EULAR criteria for RA. The first characteristic was the discriminative ability of high levels of RF compared to ACPA for early RA. Several studies observed an increased specificity for RA of a higher RF level compared to RF positivity.[5,6] However, an increased specificity for RA has also been observed for presence of ACPA compared to the presence of RF.[7] Thus far extensive comparisons of the prognostic performance for RA development of increased RF levels in comparison to the presence of ACPA, notably anti-CCP antibodies, have not been made. In three separate prospective cohorts with undifferentiated arthritis (UA) patients of recent onset from three different countries, RA development was studied in relation to baseline RF levels and ACPA. RA was defined by the 1987 ACR criteria.[1] To verify that the results were not different when other outcome measures were used, analyses in UA patients were repeated with arthritis persistency as outcome. Furthermore, in RA patients the same analyses were performed with the rate of joint destruction and the achievement of sustained disease modifying antirheumatic drugs (DMARD)-free remission as outcome.

The second characteristic was the capacity of different assays to uniformly define a high RF level. Despite the presence of international units for RF, RF level measurement is not adequately

standardized between different measurement methods. Subsequent variations in RF levels may yield differences in classifying or diagnosing RA between laboratories. Therefore we determined the degree of variation in RF levels obtained when the same RF-positive serum samples were tested by the methods that are currently most frequently applied (ELISA, nephelometry, turbidimetry). Although older studies evaluated the correlations between results of the Rose-Waaler method and ELISA,[8] data on a head-to-head comparisons of currently applied methods are to the best of our knowledge not available.

PATIENTS AND METHODS

Patients

Development of RA in UA patients

UA patients of three separate cohorts were studied for RA development, comprising an overall total of 972 UA patients (Figure 1). UA was defined as not fulfilling any of the existing classification criteria for a rheumatic disease diagnosis 2 weeks after the first presentation when the results of laboratory and radiological examinations were known.[9] Patients were followed up for one year, where after the final diagnosis was established. Patients were categorized as RA (according to the 1987 ACR criteria)[1] or as non-RA (all other diagnoses).

The Leiden EAC is a large prospective cohort that started in 1993, which has been described previously.[10] Patients with confirmed arthritis were included when the symptom duration was less than 2 years. At baseline, blood samples were taken for routine diagnostic laboratory screening (including testing for IgM-RF) and stored for determining other auto-antibodies later on (anti-CCP2). Follow-up visits (including radiographs) were performed yearly. Between 1993 and 2006, 625 patients were diagnosed with UA at baseline. Almost all patients had a follow-up duration longer than one year and 30% of the UA patients had developed RA after one year and 4% later than one year of follow-up.[11]

The Berlin EAC was started in January 2004, and patients were included if they had synovitis in at least 2 joints and a duration of symptoms between 4 weeks and 12 months. This Berlin cohort has been described previously.[12] At first presentation, 154 patients had UA. Fulfillment of the 1987 ACR criteria[1] for RA was assessed after 1 year of follow-up.

The third cohort consisted of 193 UA patients from Oslo, Norway, included in the Norwegian very early arthritis (NOR-VEAC).[13] This cohort included patients with at least one swollen joint of 16 weeks duration. During the first year patients were seen after 3, 6, and 12 months and the development of RA was classified after one year of follow-up.

In the first, data driven phase of developing the new 2010 ACR/EULAR criteria, patients from the Leiden EAC (n=213) and from the NORVEAC (n=193) were used.[3] All studies were approved by the local ethics committees. All patients gave their written informed consent.

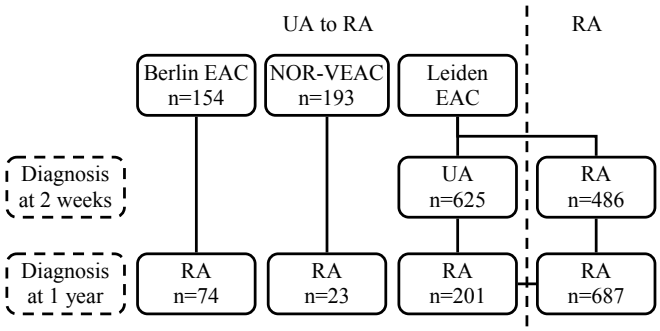

Figure 1. Flowchart of the cohorts used in this study. Left Part: Patients initially diagnosed with UA were studied for development of RA. For the Berlin data, all of the 154 UA patients had data on RF as well as ACPA. Also in the NOR-VEAC, both RF and ACPA were determined in all 193 UA patients. For the Leiden EAC, data was available for RF in 623 and for ACPA in 624 out of 625 UA patients. Right Part: In the Leiden EAC, a total of 687 patients were diagnosed with RA after one year. In these patients 686 had data on radiographic data and/or the achievement of sustained DMARD-free remission. RF and ACPA were in measured in 663 and 658 patients respectively

Arthritis persistency in UA patients

In order to determine whether results differed when another outcome measure was used, analyses were repeated with arthritis persistency as outcome in the Leiden dataset. A generally accepted definition for persistency is lacking and its frequency depends on the observation period. We defined persistent arthritis as the absence of sustained remission, which was defined as the absence of swollen joints for at least one year after cessation of eventual DMARD therapy. When remission was not obtained after 5 years of disease, a patient was classified as having persistent arthritis. With this definition, 61.3% of UA patients had persistent arthritis.

Severity of disease course in RA patients

Patients who fulfilled the ACR 1987 criteria for RA during the first year and were included in the Leiden EAC between 1993 and 2006 were studied. Of the total of 687 RA patients, 486 had already fulfilled the 1987 ACR criteria for RA at baseline and 201 developed RA within the first year of follow-up (Figure 1).

672 RA patients had radiographs of hands and feet taken at baseline and on consecutive years. These were scored chronologically by an experienced reader (MPMvdL) according to the Sharp/van der Heijde method.[14] Intraobserver intraclass correlation coefficients (ICC) were 0.91 for all radiographs, 0.84 for baseline radiographs, and 0.97 for the radiographic progression rate. To encompass a reliable sample size, radiographic follow-up data were restricted to a maximum of 7 years (median 5, IQR 2-7). Treatment strategies for RA had changed over time and became more aggressive in subsequent inclusion periods (1993-1996, 1996-1998 and 1999-2006), see reference.[15]

A second outcome measure for the severity of the disease course was the achievement of sustained DMARD-free remission. Remission was defined in a stringent form as the persistent absence of synovitis, e.g. no swollen joints, for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheumatologist.[16] Here, corticosteroids (both oral and intra-articular) were considered as DMARDs; NSAIDs were allowed. Most patients who achieved remission had a follow-up after cessation of DMARDS longer than one year. The remission status could be reliably ascertained in 641 RA patients using medical files. The frequency of DMARD-free remission in these RA patients was 12.3%.

Autoantibody testing

In the Leiden EAC, RF was determined by enzyme-linked immunosorbent assay (ELISA) (IgM-RF, in-house ELISA),[17] using a standard cutoff value of 5 arbitrary units. Anti-CCP2 autoantibodies (total IgG) were measured by ELISA (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff level for anti-CCP2 autoantibody positivity was set at 25 arbitrary units, according to the manufacturer's instructions.

In the Berlin cohort, RF was determined by ELISA (Autostat II, Hycor Biomedical, Edinburgh, UK), using a reference value of >24 IU/l Units for a positive test result. Anti-CCP 2 was determined by ELISA (Immunoscan CCPlus, Euro-Diagnostica, Malmö, Sweden), using a reference cutoff of >25 U/l for autoantibody positivity.

In the NOR-VEAC, sera frozen at inclusion were used to analyze anti-CCP2 (Inova Inc., San Diego, USA) and IgM-RF (in-house ELISA) levels in one batch. Cutoffs used to define a positive status were as recommended by the local laboratory: anti-CCP2 25 units/ml and IgM-RF 25 units/ml.

Considering the absence of agreement on a uniform definition of high level RF, two definitions of high RF level were evaluated. These were three times the reference cutoff value, the definition of a high RF level that is used in the 2010 ACR/EULAR criteria, and a RF level of 50 U/ml (RF_{50}), as RF_{50} is the definition of high RF levels used in previous studies on this subject.[5,6]

Variation in RF measurements

In order to facilitate laboratories in quality control in the Netherlands, the SKML - section HIM (Stichting Kwaliteitsbewaking Medische Laboratoria – section Humoral Immunology) organizes external quality assessment schemes for rheumatoid factor testing twice a year. In each scheme six patient samples are sent to 78 participating laboratories. These six patient samples consist of three RF-negative samples, two RF-positive samples and one standard serum (RELARES). This is a commercially available standard serum, consisting of pooled serum of RF-positive patients, which was previously standardised to correspond with 100 International Units using the Rose-Waaler agglutination test.[18,19] For this paper the results of the spring 2008 scheme are used of the two RF-positive patient sera and the standard serum. The sera were tested according to local

protocols and reported in local units and as a ratio compared to the local cutoff value by the participants.

Statistical analysis

Development of RA in UA patients

Different test characteristics (sensitivity, specificity, positive (LR+) and negative (LR-) likelihood ratio) were determined. The likelihood ratio incorporates both the sensitivity and specificity of the test and provides an estimate of how much a test result will change the odds of having a disease. In addition, absolute post test changes on RA after 1 year of follow were determined (positive predictive value (PPV) and negative predictive value (NPV)). Analyses were performed using two descriptions of a high RF level (three times the reference cutoff level and a RF level of 50 U/ml (RF_{50}), and the resulting data were compared with the data for ACPA positivity. RA development was analyzed after 1 year of follow-up and arthritis persistency was classified after 5 years of follow-up.

Severity of disease course in RA patients

Associations with the rate of joint destruction during 7 years of follow-up were assessed using a repeated measurement analysis (RMA) on log-transformed radiological data, because of skewness. The RMA is performed using a multivariate normal regression model that, on longitudinal data, evaluates the progression rates over time and takes into account the correlation between the measurements within one subject. Adjustments were made for age, gender and applied treatment strategy as previously described.[20]

Analysis of sustained DMARD-free remission was performed by comparing Kaplan Meier curves and by Cox regression analysis, correcting for age and gender, taking into account the differences in follow-up times among patients. For patients who achieved remission, the dependent variable was "time-to-event", indicating the time until reaching remission. For non-remission patients the time to last follow-up was used.

Variation in RF measurements

To test for correlations between the different methods that are used for measurement of the RF level, non-parametric Spearman correlation coefficients (ρ) were determined.

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used. P-values <0.05 were considered significant. All reported p-values are two-sided.

RESULTS

Development of RA in UA patients

Baseline characteristics of UA patients included in the three cohorts are presented in Table 1. The percentages of UA patients that developed RA within the first year were 32%, 48% and 12% in the Leiden EAC, Berlin EAC and NOR-VEAC respectively.

First, the predictive values for high RF levels and presence of ACPA antibodies were determined for each cohort separately (Table 2). Increasing the cutoff value for a high RF level yielded an increased PPV and decreased NPV. Similarly, the specificity increased but the sensitivity decreased. For example, in the Leiden EAC data, the PPV increased from 62% (RF positivity) to 69% (three times the reference value) and 72% (RF_{50}) and the NPV decreased from 78% to 75 % and 71% respectively. Also, the specificity increased from 86% (RF positivity) to 93% (three times the reference value) and 97% (RF_{50}) but the sensitivity decreased from 48% to 33% and 14% respectively. In addition, the LR+ increased at the expense of an increased LR-. This indicates that the odds on RA increased in case of a high RF level, but that the odds on RA in case of the absence of a high RF level increased as well. The percentage of UA patients that had a high RF level was 15% (three times the reference value) or 6% (RF_{50}) compared to 25% that was RF positive. The observed effects were comparable for all three cohorts (Table 2).

Second, the results for a high RF level were compared to that of ACPA positivity. In all three cohorts, the 95% confidence intervals (95% CI's) overlapped. Nevertheless the balance between PPV (preferably high) and NPV (preferably high) tended to be better for ACPA than for high level RF. In addition, the balance between LR+ (preferably high) and LR- (preferably low) was better for ACPA presence than for high RF level in all three cohorts. These effects were less compelling in the NOR-VEAC than in the Berlin EAC and Leiden EAC. However, the findings in the NOR-VEAC are more difficult to interpret because of large confidence intervals. These larger

Table 1. Baseline characteristics patients with early undifferentiated arthritis included in the different cohorts

Characteristics	Leiden EAC (n=625)	Berlin EAC (n=154)	NOR-VEAC (n=193)
Age at inclusion, (yrs)	51.0 (16.9)	51.2 (14.5)	46.1 (14.5)
Female, N (%)	368 (58.9)	110 (71.9)	114 (59.1)
Symptom duration at first presentation, days	170 (181)	137.4 (96.1)	35 (30)
Swollen joint count	5.5 (6.0)[§]	2.7 (4.5)[‡]	3.9 (6.8)[£]
CRP (mg/l), median (IQR)	17.0 (7.0-43.0)[$]	6.2 (2.0-16.8)[¥]	14.0 (5.0-32.0)[$]
RF positive, N (%)	154 (24.7)	79 (51.3)	18 (9.3)
ACPA-positive, N (%)	149 (23.9)	44 (28.6)	19 (9.8)

Values are the mean ± SD except where indicated otherwise. CRP: C-reactive protein; SJC: swollen joint count; [§]44 swollen joint count; [‡]28 swollen joint count; [£]66 swollen joint count; [$]Used cutoff for abnormal CRP ≥10 mg/l; [¥]Used cutoff for abnormal CRP >5 mg/l; RF = rheumatoid factor; ACPA = antibodies to cyclic citrullinated peptide

Table 2. Comparison of different high level cutoffs for RF and the reference ACPA for predicting progression from UA to RA in three different cohorts

Patient Cohort	Autoantibody test (cutoff value)	No. of UA patients with a positive test result (%)	PPV	NPV	Likelihood Ratio		Sensitivity	Specificity
			% (95% CI)	% (95% CI)	Pos. (95% CI)	Neg. (95% CI)	% (95% CI)	% (95% CI)
Leiden EAC n=625	RF (5.0)[§]	154 (24.8)	61.7 (54.0-69.4)	77.8 (74.1-81.6)	3.45 (2.60-4.53)	0.61 (0.53-0.70)	47.7 (40.8-54.7)	86.1 (82.8-89.4)
	RF (15.0)[‡]	96 (15.4)	68.8 (59.5-78.0)	74.9 (71.2-78.6)	4.71 (3.17-7.01)	0.72 (0.65-0.79)	33.3 (26.8-39.9)	92.9 (90.5-95.4)
	RF (50.0)[¥]	39 (6.3)	71.8 (57.7-85.9)	70.8 (67.2-74.5)	5.45 (2.77-10.72)	0.88 (0.83-0.94)	14.1 (9.3-19.0)	97.4 (95.9-98.9)
	ACPA[†]	149 (23.9)	67.1 (59.6-74.7)	78.9 (75.3-82.6)	4.33 (3.21-5.83)	0.57 (0.49-0.65)	50.0 (43.1-56.9)	88.4 (85.4-91.5)
Berlin EAC n=154	RF (24.0)[§]	54 (35.3)	68.4 (58.1-78.6)	73.3 (63.3-83.3)	2.34 (1.64-3.33)	0.39 (0.26-0.59)	73.0 (62.9-83.1)	68.8 (58.6-78.9)
	RF (50.0)[¥]	39 (25.3)	72.2 (60.3-84.2)	65.0 (55.7-74.3)	2.81 (1.70-4.66)	0.58 (0.45-0.76)	52.7 (41.3-64.1)	87.3 (72.7-89.8)
	RF (72.0)[‡]	34 (22.1)	79.1 (66.9-91.2)	64.0 (55.0-72.9)	4.08 (2.10-7.93)	0.61 (0.49-0.76)	45.9 (34.6-57.3)	88.8 (81.8-95.7)
	ACPA[†]	41 (26.6)	93.2 (85.7-100.6)	70.0 (61.4-78.6)	14.77 (4.78-45.68)	0.46 (0.36-0.60)	55.4 (44.1-66.7)	96.3 (92.1-100.4)
NOR-VEAC n=193	RF (25.0)[§]	11 (5.7)	61.1 (38.6-83.6)	93.1 (89.4-96.9)	11.61 (5.01-26.95)	0.54 (0.37-0.81)	47.8 (27.4-68.2)	95.9 (92.9-98.9)
	RF (50.0)[¥]	9 (4.7)	75.0 (50.5-99.5)	92.3 (88.4-96.2)	22.17 (6.47-76.01)	0.62 (0.45-0.86)	39.1 (19.2-59.1)	98.2 (96.3-100.2)
	RF (75.0)[‡]	6 (3.1)	85.7 (59.8-111.6)	90.9 (86.7-95.0)	44.35 (5.59-352.06)	0.74 (0.59-0.95)	26.1 (8.1-44.0)	99.4 (98.3-100.6)
	ACPA[†]	14 (7.3)	73.7 (53.9-93.5)	94.8 (91.5-98.1)	20.70 (8.22-52.12)	0.40 (0.24-0.67)	60.9 (40.9-80.8)	97.1 (94.5-99.6)

ACPA: anti-citrullinated-peptide-antibodies; RF: rheumatoid factor; UA: undifferentiated arthritis; RA: rheumatoid arthritis; PPV: positive predictive value; NPV: negative predictive value; 95% CI: 95% confidence interval. [§]Reference cutoff for RF-positivity. [‡]High RF cutoff level: three times reference level. [¥]High RF cutoff level: an absolute level of 50 U/ml. [†]Reference cutoff for ACPA-positivity

confidence intervals may be related to the low percentage of UA patients with a high RF level in this very early cohort (3% for three times the reference value and 5% for RF_{50}). When arthritis persistency was used as outcome measure instead of RA development comparable observations were made (Supplementary Table 1).

Subsequently, the additive value of performing a second autoantibody test was investigated for predicting RA development. In other words, the additive value of performing an ACPA test in UA patients without high level RF was determined, as well as the additive value of testing RF levels in ACPA negative UA patients. As shown in Table 3, the PPVs and NPVs of performing an ACPA test in patients without a high level RF were about twice as large compared to the PPVs and NPVs of RF level testing in ACPA negative patients. This observation was done for different definitions of high level RF and in the different cohorts. The LR+ for additional ACPA testing in patients without a high level RF ranged between 3.6 and 12.4 and the LR- ranged between 0.63 and 0.77 in the Leiden and Berlin EACs. RF level testing in ACPA negative patients resulted in marginal LR+ and LR- (around 1) in these cohorts. This contrast was less evident in the NOR-VEAC but also here the number of ACPA negative UA patients that developed RA that had high levels of RF was very low (n=1). Overall, for the prediction of RA development in early UA patients, performing an ACPA test in addition to a RF level testing seems more valuable than determining the RF level after assessments on the presence of ACPA antibodies.

Severity of disease course in RA patients

The predictive ability for the severity of RA was assessed and compared for high level RF and presence of ACPA. The rate of joint destruction for patients with high RF levels (both for RF_{50} and three times the reference value) and ACPA positive RA patients are depicted in Figure 2A. To compare the effect sizes of the three groups, the estimates obtained from the repeated measurement analyses performed on log-transformed data were back-transformed to the original scale. This yielded a 1.13, 1.05 and 1.04 times greater progression rate per year for the presence of ACPA, three times the reference value of RF and RF_{50} respectively compared its the absence. Over a total followup period of seven years this resulted in 2.41 (95%CI 2.06-2.83, $p<0.001$), 1.45 (95%CI 1.24-1.70, $p<0.001$) and 1.29 (95%CI 1.05-1.59, $p=0.015$) times larger progression rates for ACPA, three times the reference value of RF and RF_{50}.

To further substantiate the findings on RA severity, the analyses were performed with the achievement of sustained DMARD-free remission as outcome (Figure 2B). Presence of ACPA or high RF levels was associated with a worse disease outcome, reflected by an increased hazard ratio (HR) for not achieving DMARD-remission. The observed HRs for not achieving DMARD-free remission were respectively 11.3 (95%CI 5.6-22.7, $p<0.001$), 5.7 (95%CI 2.9-11.4, $p<0.001$) and 3.1 (95%CI 1.2-7.6, $p=0.016$) for ACPA, three times the reference value of RF and RF_{50}. Similar to joint destruction, the effect sizes for high level RF (RF_{50} as well as three times the reference value) were lower than that for the presence of ACPA antibodies.

Table 3. Additional value of testing for high level RF or ACPA in case of a negative test result for the other autoantibody test in predicting development of RA from UA

Patient Cohort	Primary Test result	Additional Test performed	PPV	NPV	Likelihood Ratio		Sensitivity	Specificity	Add. no. of RA-patients with a positive test result (%)
			% (95% CI)	% (95% CI)	Pos.(95% CI)	Neg.(95% CI)	% (95% CI)	% (95% CI)	
Leiden EAC n=625	RF_{15}-‡	ACPA†	54.3 (42.6-66.0)	79.6 (75.9-83.3)	3.57 (2.33-5.47)	0.77 (0.69-0.87)	29.0 (21.2-36.8)	91.9 (89.2-94.6)	38 (6.1)
	RF_{50}-¥	ACPA†	63.5 (54.7-72.3)	79.4 (75.8-83.1)	4.25 (3.04-5.94)	0.63 (0.55-0.72)	43.2 (35.7-50.7)	89.8 (86.9-92.7)	73 (11.7)
	ACPA-†	RF_{15}‡	23.5 (3.4-43.7)	79.6 (75.9-83.3)	1.19 (0.40-3.57)	0.99 (0.95-1.04)	4.1 (0.2-8.1)	96.5 (94.7-98.4)	4 (0.6)
	ACPA-†	RF_{50}¥	20.0 (-15.1-55.1)	79.4 (75.8-83.1)	0.97 (0.11-8.55)	1.00 (0.98-1.02)	1.0 (-1.0-3.0)	98.9 (79.9-100.0)	1(0.2)
Berlin EAC n=154	RF_{50}-¥	ACPA†	84.6 (65.0-104.2)	72.4 (63.0-81.8)	10.21 (2.40-43.52)	0.71 (0.56-0.89)	31.4 (16.0-46.8)	96.6 (92.7-101.1)	11 (7.1)
	RF_{72}-‡	ACPA†	87.5 (71.3-103.7)	72.6 (63.7-81.6)	12.43 (2.97-51.92)	0.67 (0.53-0.84)	35.0 (20.2-49.8)	97.2 (93.3-101.0)	14 (9.1)
	ACPA-†	RF_{50}¥	39.1 (19.2-59.1)	72.4 (63.0-81.8)	1.50 (0.72-3.12)	0.89 (0.70-1.12)	27.3 (12.1-42.5)	81.8 (73.2-90.4)	9 (5.8)
	ACPA-†	RF_{72}‡	46.7 (21.4-71.9)	72.6 (63.7-81.6)	2.04 (0.81-5.17)	0.88 (0.73-1.07)	21.2 (7.3-35.2)	89.6 (82.8-96.4)	7 (4.5)
NOR-VEAC n=193	RF_{50}-¥	ACPA†	54.5 (25.1-84.0)	95.3 (92.1-98.5)	14.31 (4.99-41.07)	0.59 (0.37-0.93)	42.9 (16.9-68..8)	97.0 (94.4-99.6)	6 (3.1)
	RF_{75}-‡	ACPA†	64.3 (39.2-89.4)	95.3 (92.2-98.5)	17.89 (6.76-47.34)	0.48 (0.29-0.80)	52.9 (29.2-76.7)	97.0 (94.5-99.6)	9 (4.7)
	ACPA-†	RF_{50}¥	25.0 (-17.4-67.4)	95.3 (92.1-98.5)	6.11 (0.70-53.07)	0.91 (0.72-1.14)	11.1 (-9.4-31.6)	98.2 (96.1-100.2)	1 (0.5)
	ACPA-†	RF_{75}‡	50.0 (-19.3-119.3)	95.3 (92.2-98.5)	18.33 (1.25-269.92)	0.89 (0.71-1.13)	11.1 (-9.4-31.6)	99.4 (98.2-100.6)	1 (0.5)

ACPA: anti-citrullinated-peptide-antibodies; RF: rheumatoid factor; UA: undifferentiated arthritis; RA: rheumatoid arthritis; PPV: positive predictive value; NPV: negative predictive value; 95% CI: 95% confidence interval; Add. no. of RA patients with a positive test result (%): number of RA patients identified by the second/additional test that was performed, the % is calculated with the total number of patients available in each cohort; ‡High RF cutoff level: three times reference level; ¥High RF cutoff level: an absolute level of 50 U/ml; †Reference cutoff for ACPA-positivity

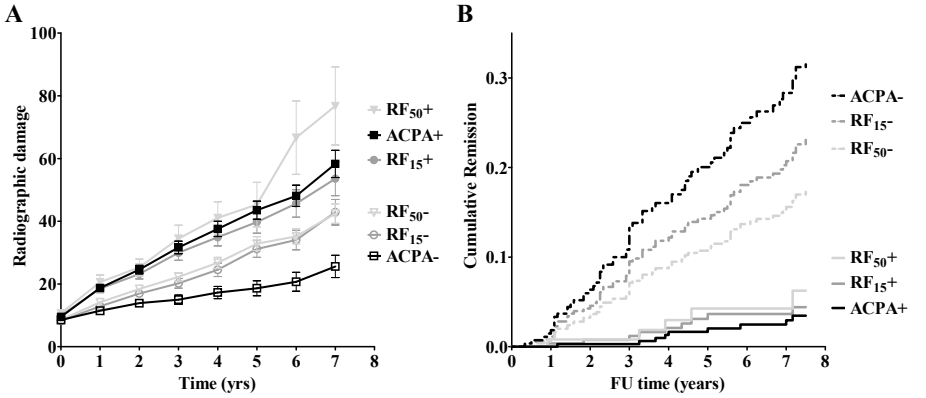

Figure 2. Comparison of high level RF and ACPA for outcome of disease severity in RA patients. Association of outcome with positive (versus negative) test results for two different high level RF cutoffs and ACPA. (A) Sharp/van der Heijde scores for radiographic progression over 7 years of followup (mean (±SEM)). (B) Achievement of DMARD-free remission. In A, the numbers of patients in each group were as follows: for RF_{15}-positive and negative, n=378 and n=271 respectively, for RF_{50}-positive and negative, n=123 and n=526 respectively and for ACPA positive and negative, n=342 and n=289 respectively. In B, the numbers of patients in each group were as follows: for RF_{15}-positive and negative, n=370 and n=252 respectively, for RF_{50}-positive and negative, n=122 and n=500 respectively and for ACPA positive and negative, n=336 and n=270 respectively. RF_{15}: three times the standard cutoff of 5.0; RF_{50}: cutoff of 50.0 U/ml; ACPA: cutoff of 25.0 arbitrary units

Variation in RF measurements

In order to evaluate whether and to what extent the method of measuring the RF level influences the test outcomes, the RF levels determined in the same serum samples by different methods were studied. The serum levels measured are shown in Figure 3A. Large variation in absolute levels was observed. In general the highest levels were measured by nephelometry, followed by turbidimetry and the lowest levels were measured by ELISA. The correlation coefficient between the absolute levels determined by nephelometry and ELISA was 0.470 (p=0.007), between nephelometry and turbidimetry was 0.531 (p=0.002) and between ELISA and turbidimetry was 0.402 (p=0.022). Since the two RF-positive sera used contained high RF levels, all of the measurements done by nephelometry and turbidimetry had an absolute RF level >50 Units. With ELISA, a measurement of <50 Units was found once. Figure 3A illustrates the large variation in measurements that is observed when local units are used.

Expressing the data as a ratio in relation to the local cutoff did not improve the variation within and between methods (Figure 3B). The correlation coefficient between these ratios was 0.288 (p=0.11) for nephelometry and ELISA, 0.443 (p=0.011) for nephelometry and turbidimetry and 0.302 (p=0.093) for ELISA and turbidimetry.

To investigate whether expression of RF level in relation to a standard reference serum would increase the reproducibility of results between laboratories and between methods, the absolute levels of the two patient sera were divided by the RF levels obtained for the standard serum (RELARES). Although the variance within the methods decreased, the variability between meth-

ods was still considerable (Figure 3C). Here, the correlation coefficients were 0.469 (p=0.008) between nephelometry and ELISA, 0.452 (p=0.012) between nephelometry and turbidimetry, and 0.537 (p=0.002) between ELISA and turbidimetry. As is shown, this effort did not lead to harmonization and reflects the difficulty with using standard sera to homogenize RF level measurements.

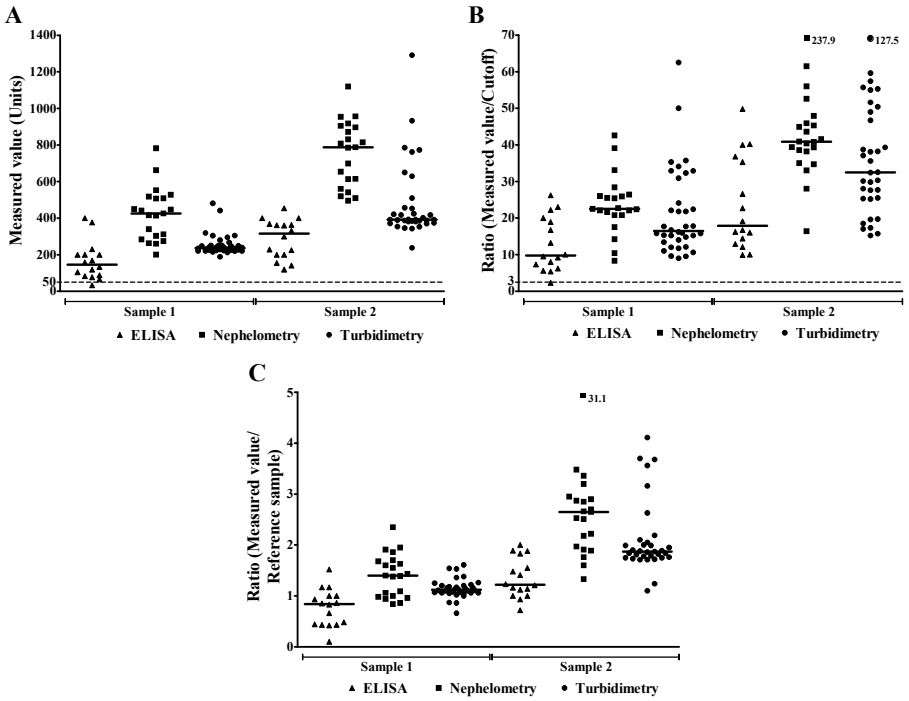

Figure 3. Comparison of RF measurements between different detection methods and different test facilities in samples positive for RF. Each dot represents a single measurement for a sample observed in a separate test facility. Horizontal bars reflect the median. (A) Units were measured in U/ml for ELISA, in kU/l for nephelometry and in IU/l for turbidimetry. The dashed line at 50 units represents the cutoff value of RF_{50}, the definition of a high RF level that is used in literature.[5,6] (B) The number of units determined by each method of measurement divided by the corresponding cutoff value. The dashed line at a ratio of 3 represents three times the reference cutoff value, the definition of a high RF level that is used in the 2010 ACR/EULAR criteria.[4] (C) The number of units determined for each method of measurement divided by the level obtained for the standard serum (RELARES) in the corresponding test facility

DISCUSSION

Detailed knowledge of the individual characteristics of the 2010 ACR/EULAR criteria is necessary to optimally use these criteria in daily clinical practice. The characteristics of the "low-positive RF" versus "high-positive RF" seem to hamper uniform application of the 2010 ACR/EULAR criteria.

The test characteristics and prognostic ability of high RF levels and the presence of ACPA were compared in early UA patients. The data, originating from three cohorts, revealed that the balance between LR+ and LR- as well as between PPV and NPV was more favorable for ACPA positivity than for high level RF. This finding was made with regards to diagnosing RA and having persistent arthritis. The same observations were done when the severity of the course of RA was studied, which substantiated the findings.

The main outcome measure used in the current study was the development of RA by fulfilling the 1987 ACR criteria for RA. An advantage of these criteria is that they could be uniformly applied in the different cohorts in Germany, Norway and the Netherlands. In the light of the new 2010 ACR/EULAR criteria however, this outcome measure may seem an outdated definition of RA. Obviously, the 2010 ACR/EULAR criteria can not be used for the purpose of the present study because of circularity; both the presence of ACPA and RF level are part of these criteria. Usage of MTX treatment as outcome measure, such as done when deriving the 2010 ACR criteria for RA, has limitations as well. UA patients in the Leiden cohort were included since 1993 and at that time DMARDs were infrequently prescribed in early UA. Hence differences in MTX prescription are dependent on the inclusion year, impairing fair comparisons. In addition, when prescribed, MTX is used for other diagnoses as well, for example psoriatic arthritis. An alternative outcome is the expert's opinion on the presence of RA. However, the expert opinion is likely not independent of the 1987 ACR-criteria for RA. Having worked with the 1987-ACR criteria for about twenty years, clinicians may, consciously or unconsciously, refer to these criteria in their judgements. In the present study, comparable observations were done when using RA development, arthritis persistency or RA severity as outcome, suggesting that the findings are not depending on one outcome measure.

Two definitions of high RF levels were studied in three cohorts. The definitions were RF_{50}, the RF level that in previous publications was labeled as high level, and three times the reference value, the definition of high RF included in the 2010 classification criteria for RA. It was observed that the post test probabilities (PPV, NPV) varied between the cohorts. For example the NPV was the highest in the NOR-VEAC and the lowest in the Berlin EAC. These values are influenced by different percentages of UA patients that developed RA during the observation period (the pretest probability). On the other hand, despite this difference, the same tendency in the level data with high RF compared to ACPA positivity was seen, strengthening the findings. The sensitivities and specificities for high RF levels differed between the cohorts as well. This may partly be due to the different cutoff levels used to define RF positivity. Subsequently, RF_{50} may be a twofold increase compared to the cutoff in some cohorts (as was the case in the Berlin EAC and the NOR-VEAC) but it may present a tenfold increase when other methods are applied (as was the case in the Leiden EAC). Although this argument may apply to a lesser extend to the three times the reference value definition for high level RF, also here the stringency with which the reference value was chosen (manufacturer instructions or according to in house reference

groups) affect the test characteristics of this variable. The differences in test characteristics of the presence of ACPA were smaller than for RF level.

Another factor that may contribute to differences in measured RF levels and differences in resulting test characteristics are the different techniques that can be used to measure RF. Here in all cohorts ELISA's were used. Generally for each technique, several variants are prevalent, among which both in house and commercially available kits. The manufacturers of these commercially available tests have not provided a 100% standardization of these kits to a reference kit with regards to detection and quantization of RF. Previously International Units/ml have been established but this method only yields standardized results in case the Boehringer nephelometer is used. The prevalent methods also differ with regard to the origin of the antibodies that are directed against RF (human or rabbit) and the isotypes of the antibodies that are tested. Nephelometry usually measure complexes of IgM-, IgG- and IgA-RF, whereas ELISAs are specifically directed against one isotype, for instance IgM-RF.

Appropriate and uniform application of the RF level criterion of the 2010 criteria for RA requires harmonization of all available RF tests. Efforts to harmonize RF determinations have been done by Dutch and European task forces. In the Netherlands this was done by the development of a standard serum consisting of pooled serum of RF-positive patients (RELARES). However, as reported, this did not result in better reproducibility between laboratories. Considerable variability was still observed, not only between various methods - ELISA, nephelometry and turbidimetry - for determining RF, but also within each method for different laboratories. Considering the present difficulties it is not feasible that worldwide harmonization will be achieved in a short term for measuring RF. This study did not address the possibility to harmonize anti-CCP level measurements. In our experience, harmonizing ACPA measurements may be less complicated (data not shown). Therefore, supposed that a modification of the 2010 ACR/EULAR criteria will arise in time, we propose to omit the RF level and use only ACPA, with different weighed scores for positivity and level.

In conclusion, defining a high level of RF is intricate due to the variation in RF levels obtained when different methods are applied. This problem hampers uniform application of the 2010 ACR/EULAR criteria for RA. The data of the present study revealed that the overall prognostic ability of ACPA positivity outweighs that of high level RF in UA patients. For this reason we suggest that in a future modification of the classification criteria for RA the RF level is not incorporated in contrast to ACPA determination.

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Supplementary Table 1. Comparison of different high level cutoffs for RF and the reference ACPA for predicting arthritis persistency in UA patients included in the Leiden EAC

Patient Cohort	Autoantibody test (cutoff value)	No. of UA patients with a positive test result (%)	PPV	NPV	Likelihood Ratio		Sensitivity	Specificity
			% (95% CI)	% (95% CI)	Pos. (95% CI)	Neg. (95% CI)	% (95% CI)	% (95% CI)
Leiden EAC n=569	RF (5.0)[§]	139 (24.4)	82.0 (75.6-88.4)	45.3 (40.6-50.1)	2.87 (1.93-4.28)	0.76 (0.70-0.83)	32.7 (27.7-37.6)	88.6 (84.4-92.8)
	RF (15.0)[‡]	86 (15.1)	86.0 (78.7-93.4)	43.1(38.6-47.5)	3.89 (2.16-6.99)	0.83 (0.78-0.89)	21.2 (16.9-25.5)	94.5 (91.5-97.5)
	RF (50.0)[¥]	35 (6.2)	85.7 (74.1-97.3)	40.3 (36.1-44.4)	3.78 (1.49-9.60)	0.94 (0.90-0.97)	8.6 (5.7-11.5)	97.7 (95.8-99.7)
	ACPA[†]	132 (23.2)	88.6 (83.2-94.1)	46.9 (42.2-51.6)	4.92 (2.95-8.19)	0.71 (0.66-0.78)	33.5 (28.6-38.5)	93.2 (89.9-96.5)

This table is based on 569 UA patients included before March 2005 to provide at least 5 years of followup for establishing persistent disease. 61.3% of these patients had persistent arthritis after 5 years. ACPA: anti-citrullinated-peptide-antibodies; RF: rheumatoid factor; UA: undifferentiated arthritis; RA: rheumatoid arthritis; PPV: positive predictive value; NPV: negative predictive value; 95% CI: 95% confidence interval; [§]Reference cutoff for RF-positivity; [‡]High RF cutoff level: three times reference level; [¥]High RF cutoff level: an absolute level of 50 U/ml; [†]Reference cutoff for ACPA-positivity

CHAPTER 5

Identification of CXCL13 as marker for outcome of rheumatoid arthritis using an *in silico* model of the rheumatic joint

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ABSTRACT

Objective

Rheumatoid arthritis (RA) is characterized by inflammation and destruction of joints. The amount of damage is highly variable between RA patients. Prediction of the disease severity using known clinical and serological risk factors is inaccurate. Here we aimed to identify new serological markers for RA severity using an *in silico* computer model of the rheumatic joint.

Methods

An *in silico* computer model of a prototypical rheumatic joint predicted candidate markers associating with erosiveness. From a broader set of candidate markers, four were progressed for validation: Trap5b, NTX, Ang-2 and CXCL13. Serum of 74 RA patients was used to study whether radiological joint destruction (total erosion score (ES) and total Sharp/van der Heijde score (SHS)) after 4-years of disease associated with serum levels at the time of diagnosis. Serum marker levels were determined using ELISAs. For confirmation, baseline serum levels were studied for an association with progression of joint damage over seven years of follow-up in a cohort of 155 early RA patients.

Results

Comparison of high and low quartiles of ES and SHS at 4-years showed a difference in baseline CXCL13 serum level ($p=0.011$ and $p=0.018$ respectively). In the confirmation cohort, elevated baseline CXCL13 levels associated with increased rates of joint destruction during 7 years follow-up, without ($p<0.001$) and with ($p<0.005$) adjustment for CRP levels. Analyzing anti-CCP2-positive and anti-CCP2-negative RA separately yielded a significant result only in the anti-CCP2-negative group ($p<0.001$).

Conclusion

CXCL13 is a novel serological marker predictive for RA severity. This marker was identified with the help of an *in silico* model of the RA joint.

INTRODUCTION

The perspectives of patients with rheumatoid arthritis (RA) have improved by treatment strategies with tight disease control and the availability of new potent biological agents. In order to balance the risks of overtreatment, inducing unnecessary costs and toxicity, and undertreatment, leading to joint destruction that could have been prevented, it is of utmost importance to be able to predict the disease outcome for each patient. At present several markers linked to a severe course of RA are extensively reported on, such as multiple swollen joints at disease onset, relatively high baseline C-reactive protein levels, presence of anti-cyclic citrullinated protein (CCP) antibodies, rheumatoid factor (RF) and erosions at baseline.[1-3] Several attempts have been made to derive adequate prediction models or prediction matrices using these risk markers, but in all cases only less than 50% of the patients could actually be classified.[4-6] This indicates that the currently used risk factors do not allow adequate prediction for individual patients. This, together with the fact that the severity of the disease course is highly variable between patients, underlines the importance to identify new markers for disease outcome in RA.

The severity of RA can be measured objectively by levels of joint destruction on radiographs of hands and feet using validated scoring methods. It reflects the cumulative burden of inflammation over time, and strongly correlates with joint functionality and subsequent disability. Several attempts to predict the radiological damage using serological markers have been made in the last few years. Examples are OPG, RANKL and MMP3 that are all primarily markers that reflect joint tissue remodeling. The baseline OPG/RANKL ratio was observed to be an independent predictor for the level of joint destruction later in the disease course.[7] Such an association is reported for baseline MMP3 levels as well, although its association was dependent on a correlation with traditional risk factors such as anti-CCP antibodies.[8] Importantly, a recent study indicated that these factors insufficiently account for radiological joint damage.[9] This underlines the value of new serological markers.

The present study aimed to identify new serological markers that are predictive for the severity of the disease course of RA. A computer model representing the biology of the rheumatic joint was used to select candidate markers for bone erosiveness. These markers were tested in baseline serum of RA patients with 4-years radiological data in order to study for an association *in vivo*. A second cohort of RA patients with long-term follow-up data was used in order to confirm the predictive ability of these markers for the disease outcome of RA, measured by the rate of joint destruction as well as the chance of sustained DMARD-free remission.

PATIENTS AND METHODS

Identification of markers

In silico model

An *in silico* computer model of a prototypical articular joint in a patient with RA was created previously (Rheumatoid Arthritis (RA) PhysioLab® platform, Entelos, Foster City, CA, USA).[10,11] This RA PhysioLab platform represents the biology behind RA on the level of synovial tissue inflammation, cartilage destruction and bone erosion. The model integrates relevant *in vitro* and clinical data into a computer-based platform to reproduce disease characteristics of RA. When run, the model simulates disease or biological response to treatment in a prototypical joint representative of affected joints of RA patients. The platform models the life cycle of inflammatory cells, endothelium, synovial fibroblasts, chondrocytes, and bone cells, as well as their products and interactions. During simulation experiments with the computer model the interplay between these cells and processes result in a reproduction of RA disease characteristics: self-perpetuating inflammation and breakdown of cartilage and bone. These characteristics are represented by numerical read-outs in the model that closely resemble accepted read-outs in the clinic, such as ACR-response, DAS28 and bone erosion progression rate. For example, bone erosion is computed as the net loss of bone volume due to bone synthesis and resorption, which in turn depend on density and activation state of osteoblasts and osteoclasts. Computed treatment responses are in line with clinically observed responses.[11] RA is a multi-factorial disease with a heterogeneous manifestation. To capture this heterogeneity the computer model uses the Virtual Patient concept. Different settings for selected (combinations of) model parameters are used in parallel for the simulations. This results in a range of disease activities and therapy outcomes. Every combination of such pre-defined parameter sets represents a Virtual Patient (VP). Examples of these settings are an increased production rate of TNFα by TNFα producing cells or a decrease of the effect of MTX on MTX-sensitive pathways in the model (see reference for a more detailed description).[12] We used RA PhysioLab version 3.2 and a set of 120 distinct VPs to predict candidate serological markers associating with localized bone loss, representing erosiveness. Therefore erosiveness is the main outcome measure used in this study. Since the erosion score is part of the Sharp/van der Heijde score, the method used to score radiological joint damage, the total SHS is assessed as well.

Patients

Discovery cohort

Patients included in the BeSt cohort for whom baseline serum was available were used to test the association of serological markers with the level of joint destruction at 4 years of followup (total erosion score (ES) and total Sharp/van der Heijde score (SHS)) (n=74). The BeSt-cohort (a

Dutch acronym for "Behandel Strategieën", treatment strategies), included patients with recent-onset RA with a disease duration of 2 years or less that were randomly allocated to one of four treatment groups with different DAS-guided combinations and applications of DMARDs.[13] At the time this study was initiated, the four years of follow-up was the maximal available follow-up duration. Applied treatments consisted of sequential disease-modifying antirheumatic drug monotherapy (group 1), step-up combination therapy (group 2), initial combination therapy with tapered high-dose prednisone (group 3), and initial combination therapy with the tumor necrosis factor antagonist infliximab (group 4) respectively.

At baseline blood samples were taken for routine diagnostic laboratory screening and serum was stored at -70 °C. There were no significant differences between baseline patient characteristics of patients with and without serum data available, apart from slightly higher numbers of swollen joints in the group with missing data (data not shown). All 4-years radiographs were scored independently by two trained readers blinded to the patient's identity, treatment group and sequence of the films. The mean score of the two readers was used for the analysis. The interobserver correlation coefficient (ICC) was 0.96.[14]

Confirmation cohort

The second set of RA patients was used to confirm findings between serological marker and severity of the disease course. This set comprised 155 early RA patients that were included in the Leiden Early Arthritis Clinic (EAC) cohort between 1993 and 2006 and for whom both baseline serum as well as yearly taken radiographs were available. No significant differences were observed between baseline patient characteristics of patients with and without serum available (data not shown). The Leiden EAC is a large prospective cohort as previously described.[15] Patients were referred by general practitioners when arthritis was suspected and included in the EAC cohort if arthritis was confirmed at physical examination and symptom duration was less than 2 years. At inclusion, patients were inquired about their joint symptoms and subjected to a physical examination. At baseline blood samples were taken for routine diagnostic laboratory screening and serum was stored at -20 °C (start of the cohort) or -70 °C. The EAC patients studied were not included in the BeSt cohort.

Radiographs of hands and feet were taken at baseline and consecutive years and were scored chronologically by an experienced reader (MPMvdL) as previously described.[16] ICCs were 0.91 for all radiographs, 0.84 for baseline radiographs, and 0.97 for the radiographic progression rate. To encompass a reliable sample size, radiographic follow-up data were restricted to a maximum of 7 years. As mentioned, the total erosion score (ES) was the main outcome measure; in addition, the total SHS was assessed. The present study had a power of 96% to detect a difference of 15 SHS points (SD25) at the seven year time point with an alpha of 0.05. Disease remission was assessed as a second outcome measure in order to further substantiate the findings. Remission was defined in its most stringent form as the persistent absence of synovitis for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheu-

matologist.[17] The remission status could be reliably ascertained in 152 out of 155 RA patients. Most patients who achieved remission had a follow-up monitoring for longer than the minimally required 1 year; the median time of observation after discontinuation of DMARDs was 2.5 years.

Biomarker measurement

Serum measurements for biomarker levels in both independent patient cohorts were performed by ELISA according to the manufacturers' instructions. Measured biomarkers were cross-linked N-teleopeptide of type I collagen (NTX) (Osteomark Ntx, Unipath Limited, Bedford, United Kingdom, 1:5 dilution), Tartrate-resistant acid phosphatase (TRAP) 5b (BoneTRAP® Assay SB-TR201A, Immunodiagnostic Systems (IDS) Ltd., Boldon, United Kingdom), Angiopoietin 2 (1:3 dilution) and Chemokine (C-X-C motif) ligand 13 (CXCL13) (both R&D systems, Minneapolis, MN, USA).

Statistical analysis

RA PhysioLab platform

RA PhysioLab version 3.2 was used for the simulations. The patient cohort consisted of 120 Virtual Patients (VPs) with different underlying pathophysiologies. Data for all VPs were analyzed after simulation of 1 year untreated disease. Erosiveness in each VP was determined by the volume of bone loss during the period of simulation. The bone loss values were categorized in quartiles and the lowest and highest quartile were compared. In total 150 simulation variables, including the concentrations of all mediators, were investigated for association to erosiveness. For the statistical analysis, Student's t, Wilcoxon rank sum and Kolmogorov-Smirnov from R version 2.4.1 were used.

Discovery cohort

To investigate whether the *in silico* model accurately predicted that erosiveness during the disease course was associated with baseline biomarker serum levels, the 4-years ES and SHS scores were categorized into quartiles. The lowest and highest quartiles were compared for differences in baseline serum level using the Mann-Whitney U test. In addition, in all RA patients the correlations between ES and SHS on a continuous scale and the serum levels were assessed using a non-parametric Spearman correlation test and a linear regression analyses on log transformed radiological data with adjustment for treatment strategy (randomization arm).

Confirmation cohort

The association between baseline CXCL13 serum levels and the rate of joint destruction during 7 years of follow-up was assessed using a repeated measurement analysis on log-transformed radiological data, correcting for age, gender and applied treatment strategy as previously described.[16] The repeated measurement analysis is performed using a multivariate normal regression model

that, on longitudinal data, evaluates the progression rates over time and takes into account the correlation between the measurements within one subject. In order to test whether biomarker levels were associated with joint destruction independent of inflammation, adjustments for C-reactive protein (CRP) were made as well. To test for associations between baseline CXCL13 levels and baseline clinical characteristics, analyses were performed using the non-parametric Spearman correlation test.

Analysis of sustained DMARD-free remission was performed by comparing Kaplan Meier curves and by Cox regression analysis, correcting for age and gender, taking into account the differences in follow-up times among patients. For patients who achieved remission, the dependent variable was "time-to-event", indicating the time until reaching remission. For non-remission patients the time to last follow-up was used.

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used. P-values <0.05 were considered significant. All reported p-values are two-sided.

RESULTS

RA PhysioLab prediction results

After simulation of one year of untreated disease the virtual patient cohort was categorized on a numerical read-out representing erosiveness. For the identification of candidate biomarkers we focused on model variables related to proteins for reasons of biomarker detection feasibility. Other variables like those related to cell densities were not taken into account for the analyses. We identified a set of proteins of which the concentrations were significantly different between the lowest and highest erosiveness quartiles in each of three statistical tests (Student's t, Wilcoxon rank sum, Kolmogorov-Smirnov; p-value < 0.0001). Four of these mediators were selected for follow-up: Ang-2, NTX, Trap5b and CXCL13. Selection of these 4 mediators was based on pragmatic criteria: assay availability and presence (NTX, Trap5b) or absence (Ang-2, CXCL13) of supportive literature linking the protein to bone erosion. The ability of CXCL13 to differentiate between high and low erosive virtual patients is illustrated in Figure 1. In the RA PhysioLab platform, most mediators are tracked as synovial quantities. For the four mediators selected transport between synovium and serum is modeled only for NTX. For Ang-2, Trap5b and CXCL13 the difference in mediator concentration between the erosiveness quartiles relates to synovial tissue concentrations; for NTX to synovial tissue and serum concentration.

Identification and replication in two independent cohorts

Baseline characteristics

Baseline characteristics of the two sets of RA patients are presented in Table 1.

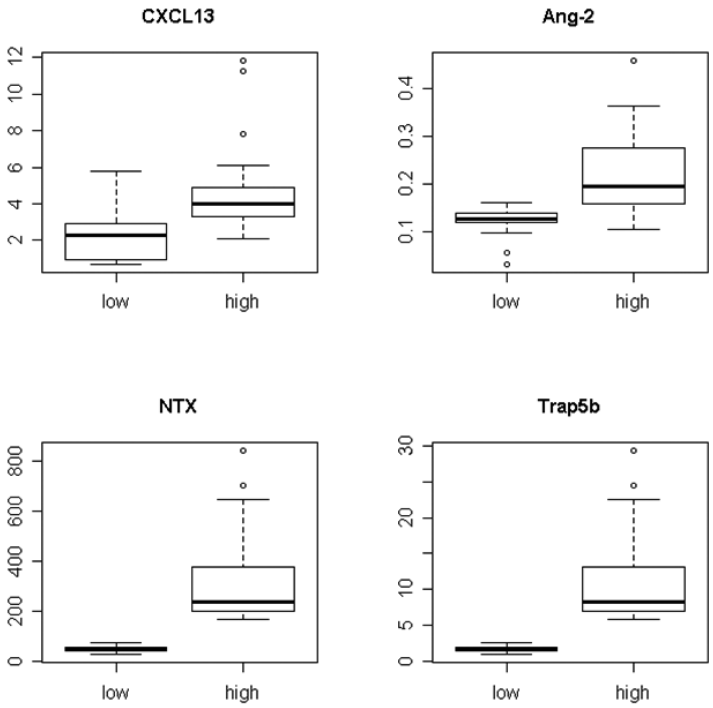

Figure 1. Boxplots of simulated synovial concentrations tested across 120 distinct Virtual Patients. For each mediator the synovial concentrations (ng/ml) are shown in the low and high erosive groups as defined in the text. The box indicates the lower and upper quartiles, the whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box

Table 1. Patient characteristics at baseline

	Discovery cohort (n=74)	Replication cohort (n=155)	p-value
Age at inclusion (yrs), mean (SD)	53.5 (14.4)	56.4 (13.6)	0.232
Female, N (%)	45 (60.8)	108 (69.7)	0.184
SJC, mean (SD)	9.27 (3.89)	8.95 (6.78)	0.107
CRP (mg/l), mean (SD)	32.0 (40.0)	31.0 (36.5)	0.774
Anti-CCP2-positive, N (%)	42 (56.8)	89 (58.2)	0.840

CRP: C-reactive protein; SJC: 66-swollen joint count. The p-value reflects if both cohorts are significantly different for the indicated variable

Discovery cohort

To study the results on markers and erosiveness predicted by the *in silico* model for an association *in vivo*, baseline serum levels for four biomarkers predicted *in silico* were determined. The mean (SD) levels were 4658.4 (1596.0) pg/ml, 51.9 (24.5) nM BCE/l, 2.19 (1.14) U/l and 166.7 (86.0) pg/ml respectively for Ang-2, NTX, Trap5b and CXCL13.

To analyze whether these biomarkers accurately predicted that erosion scores and the total level of joint destruction during the disease course were associated with baseline serum levels, the lowest and highest quartiles of 4-years erosiveness were studied in relation to the markers' serum levels (Table 2). This revealed a significant difference for CXCL13 (p=0.011 for the total erosion score (ES) and p=0.018 for the total Sharp/van der Heijde score (SHS). Similarly, significant correlations between the CXCL13 levels and the ES (p=0.022, ρ=0.267) and the SHS (p=0.014, ρ=0.286) were observed when performing the analysis on continuous data of all RA patients. In addition, analyzing joint destruction data in all RA patients using linear regression analysis revealed that baseline CXCL13 levels remained significantly associated with the 4-year ES and SHS (β=1.002 (95%CI 1.000-1.005), p=0.049 and β=1.002 (95%CI 1.000-1.005), p=0.033 respectively) after adjustment for treatment strategy. For Ang-2, NTX and Trap5b, no significant associations were observed (Table 2). Taken together these data indicate that out of 4 serum markers predicted by the *in silico* model one marker was actually observed to associate with joint destruction in patients using the analysis workflow described.

Table 2. Baseline marker serum levels compared for low and high ES and SHS at 4 years of disease duration

		Discovery cohort (n=74)			
		Ang-2 (pg/ml), mean (SD)	NTX (BCE/l), mean (SD)	Trap5b (U/l), mean (SD)	CXCL13 (pg/ml), mean (SD)
4 year ES	1st quartile	4361.2 (1401.9)	48.47 (16.41)	2.45 (0.94)	137.7 (80.4)
	4th quartile	5286.8 (1611.8)	62.89 (40.66)	2.61 (1.41)	189.8 (66.1)
	p-value	0.121	0.548	0.717	0.011
4 year SHS	1st quartile	4444.0 (1606.7)	51.61 (15.64)	2.41 (0.99)	139.6 (80.6)
	4th quartile	5279.2 (1634.5)	55.83 (23.23)	2.08 (1.32)	186.9 (64.9)
	p-value	0.190	0.800	0.373	0.018

ES: Erosion score; SHS: Total Sharp-van der Heijde score. Note that the SHS is composed of erosion and joint space narrowing scores. Differences in levels between quartiles 1 (low) and 4 (high) were compared using the non-parametric Mann-Whitney U test

Confirmation cohort

For confirmation, baseline serum CXCL13 levels were measured in a second set of patients, yielding a mean concentration of 155.5 (98.9) pg/ml. Baseline serum levels were categorized as low or high based on quartile distribution and were studied in association with the rate of progression in joint destruction over 7 years. Using repeated measurement analysis, higher CXCL13 levels associated with significantly higher progression rates in ES (p<0.001) as well as in SHS (p<0.001; analyses performed on log-transformed data) (Figure 2). For the erosion score, the increase per year in the original scale was 1.12, 1.08 and 1.18 times greater for CXCL13 quartiles 2, 3 and 4, respectively, than for quartile 1. For the total SHS, a 1.11, 1.09 and 1.18 times greater increase per year was observed compared to the lowest quartile. Over a period of 7 years this resulted in

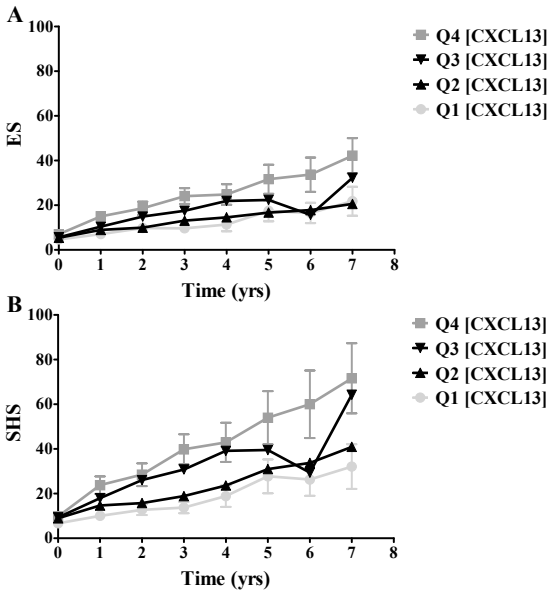

Figure 2. CXCL13 level and progression in radiographic joint damage in RA patients in the confirmation cohort. Erosion (panel A: ES) and Sharp/van der Heijde (panel B: SHS) scores (n=155). The number of patients and levels (mean (SD)) for CXCL13 were n=37, 59.2 (15.9) pg/ml, n=40, 107.6 (13.8) pg/ml, n=39, 158.9 (17.4) pg/ml and n=39, 292.3 (90.9) pg/ml for quartiles 1, 2, 3 and 4 respectively

2.2 (95%CI 1.5-3.3), 1.8 (95%CI 1.2-2.6) and 3.1 (95%CI 2.1-4.7) times larger progression rates for the ES and 2.1 (95%CI 1.4-3.3), 1.9 (95%CI 1.2-2.8) and 3.2 (95%CI 2.1-4.9) times larger progression rates for the SHS. Since categorical analysis generally results in less discriminative ability, the CXCL13 level was also included in the repeated measurement analysis as a continuous variable. Also here, higher CXCL13 levels associated significantly with higher progression rates of ES and SHS (both $p<0.001$). This analysis was also used to determine the variance explained by CXCL13. This showed that 7% of the total variance in progression in ES was explained by CXCL13.

Clinical associations of CXCL13

To study clinical factors that possibly influenced the observed association of CXCL13 with the rate of joint destruction in the confirmation cohort, baseline patient characteristics were analyzed in relation to baseline CXCL13 serum levels. Significant correlations were found for CXCL13 level and CRP level ($p<0.001$, $\rho=0.429$), ESR level ($p=<0.001$, $\rho=0.300$) and the number of swollen joints ($p=0.023$, $\rho=0.255$). In addition, mean CXCL13 levels were significantly higher in serum samples from anti-CCP2 positive patients than anti-CCP2 negative patients (172.0 (104.7) pg/ml vs.134.8 (87.3) pg/ml, $p=0.008$). Similar results were observed for IgM-RF, yielding levels of 172.9 (104.8) pg/ml vs. 120.2 (77.7) ($p<0.001$) for IgM-RF positive compared to IgM-RF negative patients respectively.

CXCL13 in relation to other serological markers

It was studied whether CXCL13 serum levels associated with ES and SHS independently of the known serological markers CRP and anti-CCP2. First CRP was entered as adjustment variable in the repeated measurement analysis. Also here CXCL13 was significantly associated with the ES (p=0.001) and the SHS (p=0.004). In addition, since anti-CCP2 positive and anti-CCP2 negative RA are considered to be separate subsets of the disease with differences in underlying pathogen mechanisms, the mentioned analyses were repeated in the anti-CCP2+ and anti-CCP2− subsets. In anti-CCP2 negative patients, high CXCL13 levels were also significantly associated with larger ES and SHS progression rates (p<0.001 and p=0.001 respectively). After adjustment for CRP level, the association remained significant for the ES (p=0.002), but significance was lost for the SHS (p=0.10). In anti-CCP2 positive patients no associations were observed for either the ES or SHS (data not shown).

Sustained DMARD-free remission

To further substantiate the CXCL13 findings we investigated a different outcome measure for RA severity and studied the effect of CXCL13 level on the achievement of DMARD-free remission. In addition to an observed association of CXCL13 levels and joint damage, comparison with the achievement of remission showed that higher CXCL13 levels were associated with significantly lower chances of achieving remission. Compared to the first quartile CXCL13, hazard ratios of 3.3 (95%CI 1.0-11.1) (p=0.049), 4.1 (95%CI 1.1-14.8) (p=0.034) and 6.3 (95%CI 1.4-29.2) (p=0.019) for not achieving remission were observed for the 2nd, 3rd and 4th quartile of CXCL13 respectively (Figure 3). With an additional adjustment for CRP, the hazard ratios were respectively 4.1 (95%CI 0.8-21.2), 2.6 (95%CI 0.7-10.2) and 2.6 (95%CI 0.7-9.0). Because only 3 patients achieved remission in the anti-CCP2 positive RA group, no stratified analysis was performed here.

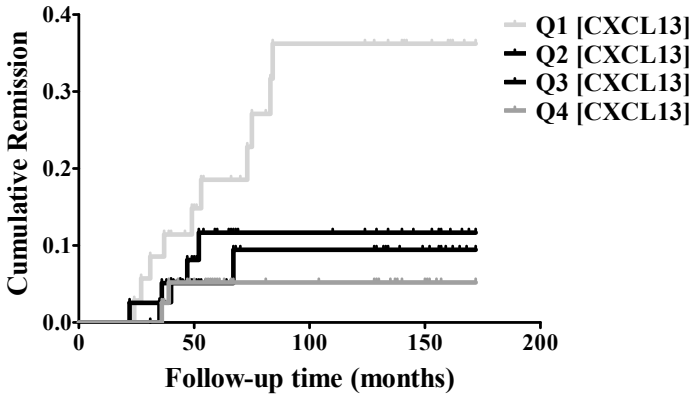

Figure 3. CXCL13 level and cumulative DMARD-free remission in RA patients in the replication cohort. The number of patients and levels (mean (SD)) for CXCL13 were n=35, 60.0 (15.9) pg/ml, n=39, 107.2 (13.7) pg/ml, n=39, 158.9 (17.4) pg/ml and n=39, 292.3 (90.9) pg/ml for quartiles 1, 2, 3 and 4 respectively. In total 19 patients achieved DMARD-free remission

DISCUSSION

The heterogeneous nature of RA severity is presently incompletely understood and hampers accurate disease prognoses on the individual patient level. This underlines the need for new disease markers with improved prognostic potential of marker sets in order to guide adequate treatment regimes. Also, exploration of new markers will enhance our understanding of involved pathophysiological processes. Exploration of suitable new markers was initiated by candidate marker prediction using the RA PhysioLab computer model of the rheumatic joint. The value of PhysioLab simulation approaches has been shown previously in RA[11] as well as in other diseases.[18-20] The PhysioLab simulation platform allows to perform question based simulation experiments from which the results contribute to hypotheses driven, focused follow-up experimental research. For this approach it is assumed that the scope of the computer model resembles real disease and patient population behavior as well as possible. In this study we identified a new biomarker, CXCL13, which showed an association between baseline serum level and the level of joint destruction at 4 years of disease, thereby validating the association predicted by the *in silico* model. Moreover, in an independent cohort relatively high serum CXCL13 levels were also associated with an enhanced progression of the rate of joint destruction over 7 years and a decreased chance of achieving DMARD-free remission, thereby confirming the association between CXCL13 and disease severity.

The cytokine CXCL13 is also known as B lymphocyte chemoattractant (BLC) or B cell-attracting chemokine 1 (BCA-1) and is part of the CXC chemokine family. One of the main effects of CXCL13 implemented in the RA PhysioLab platform is on B cell recruitment, thus supporting a mechanism directly dependent on B cells. CXCL13 serum levels were found to be significantly higher in the serum of RA patients as compared to healthy controls.[21,22] CXCL13 serum levels were also reported to respond to therapeutic intervention with anti-TNFα therapy.[12] Evidence for joint localization of CXCL13 was found, both by the detection of mRNA in inflamed synovial tissue[23] as well as the presence of ectopic lymphoid follicles expressing CXCL13 in the synovium of chronic RA patients.[24] CXCL13 has been reported to attract B lymphocytes and to interact with the receptor CXCR5, which is expressed by B cells as well as follicular B helper T cells.[25-28] High levels of CXCR5 were also found on human osteoblasts and activation by its ligand CXCL13 induced the release of extracellular matrix degrading enzymes. As such, CXCL13 may play an important role in the process of bone remodeling.[29] These data suggest that CXCL13 may have an effect on joint damage in RA that is both dependent and independent of promoting effects on B cells.

Since CXCL13 attracts B cells and CXCL13 levels are reported to be higher in autoantibody positive RA, which was also observed in the present study, and since anti-CCP2 positive and negative RA are subsets of RA with possible differences in the pathogenesis, the effect of CXCL13 was evaluated for anti-CCP2 positive and negative RA separately. In the anti-CCP2 negative group CXCL13 associated significantly with the progression of joint destruction. Adjustment

for baseline CRP levels did not alter this association for the ES, supportive of a CXCL13 effect on joint destruction that is independent of the level of inflammation as expressed by the level of CRP. In anti-CCP2 positive RA, CXCL13 was not independently associated with progression in joint damage. Thus, from a clinical perspective, information on baseline CXCL13 levels seems most valuable in the anti-CCP2-negative sub-population of RA patients.

Analysis of the lowest and highest quartiles of erosiveness in the discovery cohort did not reveal a significant difference for Ang-2. However, when comparing the upper two quartiles with the lower two quartiles, a significant difference was found for Ang-2 (data not shown). This indicates that a second candidate marker from the four markers tested might be of interest for further exploration. In the RA-PhysioLab platform Ang-2 and CXCL13 both have an effect on endothelial lifecycle, which affects all recruitment processes.

To the best of our knowledge this is the first time that a new serum marker for RA was confirmed that was initially predicted by a computer model simulation of the rheumatic joint. This finding illustrates that the present computer model has predictive potential and may be applied to other disease outcomes.

Although the results presented in this manuscript provide a solid foundation for our conclusions, this study also has limitations. Both the discovery and confirmation cohorts consisted of a limited number of patients, which may result in limited power to accurately reach proper conclusions. As a result only relatively large differences in effect sizes may be detected and smaller effects could be missed. A strong association between CXCL13 levels and radiologic progression was observed in two separate cohorts. For Ang-2 the evidence is less conclusive than for CXCL13. Failure to detect an *in vivo* association for the other candidate biomarkers that were tested (NTX, Trap5b) despite their known association to bone biology could indicate that their behavior in a heterogeneous clinical population is not predictive.

Recently, draft validation criteria for a soluble biomarker to be regarded as a valid biomarker reflecting structural damage in RA have been established.[9,30,31] These criteria provide guidance to the types of studies needed to demonstrate the value of CXCL13 as a marker in clinical practice. Our present data reveal that it is a serological marker with a potent CRP-independent predictive value for long-term outcome in RA, thereby providing a rationale for further exploration.

In conclusion, the RA PhysioLab simulation platform has helped in the identification of CXCL13 as a new serological marker for severity of RA as measured by the long-term joint destruction and the achievement of DMARD-free remission in two independent cohorts of RA patients.

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PART III

Genetics in risk prediction of RA development and severity

CHAPTER 6

Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis

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ABSTRACT

Objective

Two novel genetic polymorphisms on chromosome 6q23 are associated with susceptibility to rheumatoid arthritis (RA). Both polymorphisms (rs6920220 and rs10499194) reside in a region close to the gene encoding tumor necrosis factor α-induced protein 3 (*TNFAIP3*). *TNFAIP3* is a negative regulator of NFκB and as such involved in inhibiting TNF-Receptor mediated signalling effects. Interestingly, the initial associations were detected in patients with long-standing RA. However, no association was found for rs10499194 in a Swedish early arthritis cohort. As this could be caused by overrepresentation of patients with severe disease in cohorts with long-standing RA, we analyzed the effect of the 6q23 region on the rate of joint destruction.

Methods

Five single nucleotide polymorphisms (SNPs) in 6q23 were genotyped in 324 Dutch patients with early RA. Genotypes were correlated to progression of radiographic joint damage for a follow-up time of 5 years.

Results

Two polymorphisms (rs675520 and rs9376293) associated with severity of radiographic joint damage in ACPA+ patients. Importantly, the effects were present after correction for confounding factors such as secular trends in treatment.

Conclusions

Our data associate the 6q23 region with the rate of joint destruction in ACPA+ RA.

INTRODUCTION

Recent whole genome association scans have revealed novel genetic polymorphisms associated with susceptibility to ACPA+ RA.[1,2] Among those, two single nucleotide polymorphisms (SNPs), rs6920220 (A allele) and rs10499194 (C allele), were found to independently associate with ACPA+ disease. Both SNPs map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes are oligodendrocyte lineage transcription factor 3 (*OLIG3*) and tumor necrosis factor α-induced protein 3 (*TNFAIP3*). The latter is of potential importance to RA pathogenesis, as the protein TNFAIP3 acts as a negative regulator of NF-κB.[3] So far, however, functional relevance of the reported polymorphisms is unknown.

Rs6920220 was initially identified in ACPA+ RA patients (minor allele OR 1.38) originating from the United Kingdom (UK).[1] It was further replicated in an extended UK-based case-control study.[4] Rs10499194 was initially identified in North American ACPA+ patients (the Brigham Rheumatoid Arthritis Sequential Study, BRASS; minor allele OR 0.67).[2] Replication was successful in two additional US cohorts selected from the North American Rheumatoid Arthritis Consortium (NARAC). Replication failed, however, in ACPA+ patients of a Swedish population-based inception cohort (the Epidemiological Investigation of Rheumatoid Arthritis cohort, EIRA).[2] This latter finding is of interest, as both BRASS and NARAC are cohorts of patients with long-standing RA (mean disease duration BRASS: 15.4 ± 12.8 years;[5] NARAC: 14.3 ± 11.1 years).[6] The EIRA study, however, was designed to identify incident cases of RA as soon as possible after disease onset, resulting in an estimated mean disease duration at inclusion of only 10 months.[7]

Association of a genetic polymorphism in cohorts of patients with longstanding disease but absence of this association in an early arthritis cohort led us to hypothesize that the 6q23 region would associate with disease severity in ACPA+ patients. Very little information is currently available on the effects of genetic variation on outcome measures in RA.[8] Therefore, we genotyped five SNPs in a Dutch early arthritis cohort (the Leiden Early Arthritis Clinic, EAC) and correlated genotyping data to progression of radiographic joint damage for a maximum follow up of 5 years.

PATIENTS AND METHODS

Patients

The Leiden EAC is a population-based inception cohort that includes patients with self-reported symptom duration of ≤2 years.[9] DNA samples of 324 patients consecutively included between 1993 and 2003 were used for analysis. For further details see supplementary file 1.

SNP selection and genotyping

Five SNPs (rs1878658, rs675520, rs9376293, rs10499194 and rs6920220) were selected based on a haplotype analysis across the 6q23 locus published previously.[2] All SNPs are in imperfect linkage disequilibrium to one another (supplementary table 1). Genotyping was performed using pre-designed Taqman allelic discrimination probes (Applied Biosystems). Each 384 well plate contained 10 ng sample DNA per well and at least 8 negative and 6 positive controls. Genotype calls and clusters were manually checked for discrepancies and doubtful calls were rejected. No SNP deviated from Hardy-Weinberg equilibrium. Genotyping call rates were 96.5 % (rs1878658), 98 % (rs675520), 95 % (rs9376293), 94 % (rs10499194), and 98.1 % (rs6920220).

Serology and radiographs

Serum samples were tested for citrulline-specific IgG antibodies using a commercially available ELISA kit (Immunoscan Mark2, Eurodiagnostica, The Netherlands). Radiographs were scored according to the Sharp/van der Heijde method[10] with known time order by one blinded, independent trained reader (supplementary file 1).

Statistical analysis

Association between genotypes and radiographic scoring data was analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL). P-values <0.05 were considered significant. All p-values reported are two-sided.

Two approaches were chosen for statistical analysis. First, the average increase in Sharp/van der Heijde scores during the follow-up period was estimated per person by regression analysis. Subsequently, the average increase (slope) of scores per genotype was compared non-parametrically using the Mann-Whitney rank-sum test.

We observed an influence of the time of inclusion (1993-2003) on the progression of radiographic joint damage reflecting most likely an improvement of treatment intensity during this 10 year time period. In order to account for this effect, we performed, as a second approach, a mixed model analysis described in detail in supplementary file 1.

RESULTS

Radiographic scores of 324 Dutch RA patients (181 ACPA+, 143 ACPA-) were available for analysis. At least five radiographic follow-up observations were available in 57% of patients. A dominant model was chosen for analysis, as the frequency of patients homozygous for the minor allele of rs1878658 (G), rs10499194 (T) and rs6920220 (A) was ≤5%. Figure 1 depicts the influence of genotypes on radiographic joint damage. ACPA+ and ACPA- subgroups were analyzed separately. Median scores and interquartile ranges (IQR) are provided for ACPA+ patients in table 1 (for ACPA- patients see supplementary table 2).

Table 1: Median Sharp van der Heijde scores (M) and interquartile ranges (IQR; 25 - 75% percentiles) per genotype for ACPA+ RA patients (# = number of patients). Genotypes were combined for rs1878658 (G), rs10499194 (T) and rs6920220 (A), as the frequency of patients homozygous for the respective minor allele was ≤5% for these SNPs

	Year																	
	0			1			2			3			4			5		
ACPA+	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#
rs1878658																		
AA	6	2 - 13	126	14	6 - 24	111	18	10 - 32	113	22.5	11 - 42	98	26.5	14 - 51.5	86	32	14 - 51.5	89
AG/GG	3	1 - 7	40	12	5.5 - 20.5	40	17	6 - 32	39	30	11.5 - 56.5	34	32	16 - 69	31	27	17 - 74	31
rs675520																		
AA	9	4.5 - 13	33	14	6.5 - 21	32	15	8 - 27.5	33	19	9 - 34	27	22	13.5 - 39.5	24	20	12.5 - 47.5	21
AG	5	1 - 10	83	12	5 - 21	73	18	9.5 - 32.5	78	25	11.5 - 54	68	32.5	14 - 61	58	33	17 - 62.5	66
GG	5	2 - 11	51	14	7 - 27	48	18	8 - 37	43	26	14 - 48	39	28	16 - 63	37	32	20 - 64	35
AG/GG	5	2 - 10	134	13	6 - 24.5	121	18	9.5 - 33	121	26	12 - 53	107	32	15 - 61	95	32	18.5 - 61	101
rs9376293																		
CC	6.5	3 - 13.5	28	15	9 - 28	27	27	12 - 49.5	25	33	15.5 - 58	24	35	16.5 - 62	24	35	16.5 - 56	21
CT	4	1 - 9	84	12	5 - 21	79	17	7 - 32.5	78	21	10.5 - 54	66	32	14 - 68	57	36	20 - 68	61
TT	8	3 - 13	54	14	6 - 21	46	16	10.5 - 25.5	50	21	12 - 30.5	44	23	13.5 - 38	38	23	13 - 47	40
CC/CT	4	2 - 10	112	13	6 - 26.5	106	18	8 - 36	103	28	11 - 54.5	90	34	15 - 63	81	36	19 - 67.5	82
rs10499194																		
CC	7	2 - 12.5	90	14	6 - 21.5	77	18	9.5 - 28	82	21	12 - 35	71	25.5	14 - 45	62	29	14 - 48	62
CT/TT	4	2 - 7.5	65	12	6 - 24.5	64	17	7 - 37	61	31	11 - 55	55	29.5	14.5 - 67	50	32	15 - 68	51
rs6920220																		
AA/AG	7	2 - 12	58	14	6 - 21	53	20	10 - 32.5	57	24	12 - 48	47	32	18 - 56	35	35.5	18 - 65	42
GG	4.5	2 - 10.5	108	13	6 - 25	99	16.5	8 - 30.5	96	22	11 - 48	86	25	14 - 61	84	24	14 - 51	79

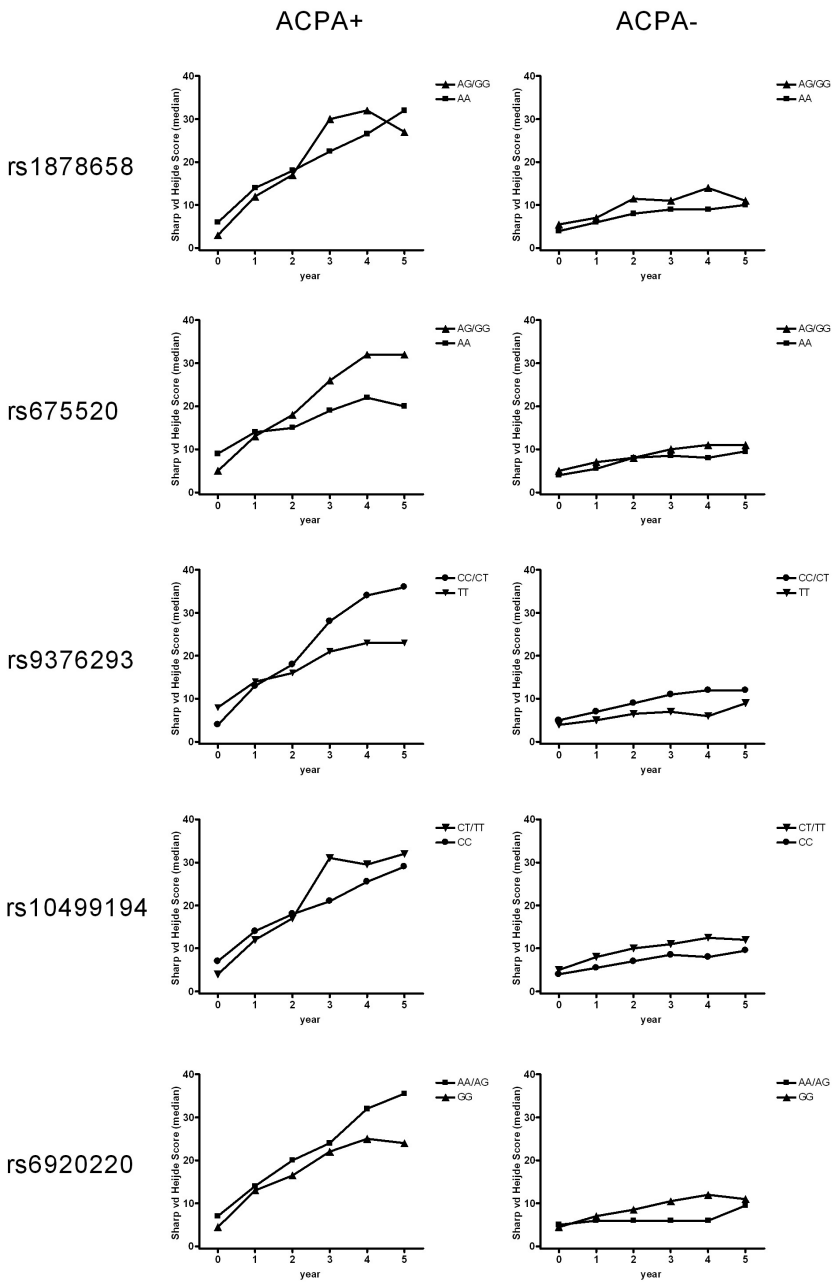

Figure 1. Development of median Sharp van der Heijde scores plotted according to genotype/allele in ACPA+ (left column) and ACPA- (right column) RA patients. Year 0 equals baseline values. Regression analysis was performed in order to estimate the average increase (slope) in Sharp van der Heijde scores over time. Slopes were subsequently compared using the nonparametric Mann-Whitney test (for the ACPA+ subgroup: p = 0.37 (rs1878658); p = 0.007 (rs675520); p = 0.021 (rs9376293); p = 0.05 (rs10499194); p = 0.76 (rs6920220))

No influence of genotypes on radiographic joint damage was observed in ACPA- patients (Figure 1). In ACPA+ patients, however, two polymorphisms showed reproducible association with disease progression over time. Presence of the G allele of rs675520 was found to associate with increased Sharp/van der Heijde scores, as a significant difference was observed when the average increase (slope) in radiographic scores over time was compared with G as the dominant allele (median slope AG/GG = 4.6, AA = 2.3; Mann-Whitney p = 0.007). In order to account for an effect of improving treatment strategies on radiographic progression during the 10 year period in which patients were included into the study, we next performed a mixed model analysis. This analysis identified the year of inclusion as a significant variable influencing the extent of radiographic joint damage (p = 0.005). After correcting for the year of inclusion, however, we still observed a significant influence of the G allele of rs675520 (AG/GG vs. AA, p = 0.026).

Similar to the G allele of rs675520, we noted an influence of the C allele of rs9376293 on progression of radiographic joint damage (Figure 1). The average increase (slope) in Sharp/van der Heijde scores over time was significantly higher for C allele carriers as compared to T homozygotes (median slope CC/CT = 4.5, median slope TT = 3.0, Mann-Whitney p = 0.021). After correcting for the year of inclusion as described above a trend effect of the C allele remained (p = 0.097).

For rs1878658, rs10499194 and rs6920220, no significant influence of individual genotypes on radiographic joint damage was noted.

DISCUSSION

The 6q23 region has recently been associated with disease susceptibility in RA. This region contains no known transcripts. The closest genes with known function are *OLIG3* and *TNFAIP3*. *TNFAIP3* encodes protein A20, a TNF-α induced negative regulator of NF-κB.[3,11] Decreased levels of A20 lead to uncontrolled NFκB-activity, resulting in increased inflammation. This observation makes *TNFAIP3*/A20 and the 6q23 region interesting candidates that could modulate inflammation also in RA.

We were intrigued by recent differential findings for rs10499194, a SNP on chromosome 6q23 close to *TNFAIP3*, in cohorts with differing disease duration. The major allele (C) was found to associate with disease susceptibility in ACPA+ RA patients in three cohorts with long-standing disease, but not in an early arthritis cohort.[2] This indicated a potential impact of the 6q23 region on disease severity. In order to test for such an impact, five SNPs were genotyped in a cohort of Dutch patients with early RA. These SNPs had previously been shown to identify common haplotypes in 6q23.[2] We identified two SNPs for which presence of alleles was associated with increased joint destruction in ACPA+ patients. Carriers of the G allele of rs675520 developed increased Sharp/van der Heijde scores over time. A similar effect, although weaker, was found for the C allele of rs9376293. Interestingly, no association was found for any of the SNPs in ACPA- individuals. Although this does not exclude a contribution of the 6q23 region to disease severity

in ACPA- disease, the latter observation is in line with recent reports detecting an association of the 6q23 region with disease susceptibility in ACPA+ patients only.[4] No effect on disease severity was observed for rs10499194 and rs6920220. Based on our data we cannot rule out the possibility that either SNP exerts a weak effect that requires larger sample numbers for detection or that cannot be observed during the first years of disease. Interestingly, we observed nominally higher scores for the riskconferring A allele of rs6920220 without reaching statistical significance. The discrepancy between SNPs associating with susceptibility and radiographic progression also indicates that the causal variant at this locus has not yet been identified. Given the large area of linkage disequilibrium surrounding these SNPs, further fine-mapping and functional characterization will have to be performed.

Data linking newly identified genetic polymorphisms to disease outcome in RA are only beginning to emerge. Our data are unique, as they cover a long period of radiographic follow-up and have been scrutinized for artefacts such as secular trends in treatment intensity. Albeit based on relatively low patient numbers, our data indicate a contribution of the 6q23 region to the rate of joint destruction in ACPA+ RA, thereby further refining our understanding of the effects exerted by this locus. Replication of our findings in other cohorts is needed. Nonetheless, this is the first study demonstrating such an effect for genetic polymorphisms located outside the HLA-region in ACPA+ RA patients.

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SUPPLEMENTARY INFORMATION ON PATIENTS AND METHODS

Patients

All patients met the American College of Rheumatology 1987 revised classification criteria for RA and were of Caucasian origin based on self-reported ethnicity. Written informed consent was obtained from all participants, and the study was approved by the local institutional review board.

The Leiden Early Arthritis Clinic (EAC) is a population-based inception cohort that includes patients with self-reported symptom duration of ≤2 years. Follow-up visits are performed and radiographs of hands and feet are taken on a yearly basis. DNA samples of 324 patients (67.6% female; mean age 56.3 ± 15.4 years) consecutively included between 1993 and 2003 for whom radiographic scoring data and ACPA status were available were used for analysis. 2003 as the latest year of inclusion was chosen in order to allow a five year followup period for all patients.

Radiographic scoring

The number of patients with available radiographs varied per time-point (for ACPA+ patients: n = 168 at baseline and n = 153, 154, 134, 119, and 122 at year 1 to 5, respectively; for ACPA- patients: n = 135 at baseline and n = 121, 109, 93, 81, and 65 at year 1 to 5, respectively). In total, radiographs of 324 patients (181 ACPA+, 143 ACPA-) were used for analysis. All radiographs were scored by one experienced reader who was blinded with respect to the patient's autoantibody status, treatment, clinical outcome and genotyping results. Scoring was performed with known time order, which is more sensitive to change compared to scoring with unknown time sequence.[1] For quality control, radiographs of 60 randomly selected RA patients were rescored by the same reader. This selection comprised 499 radiographs, consisting of 149 baseline radiographs and 350 radiographs during followup. Reliability of radiographic scoring was calculated. Intraclass-observer correlation coefficients (ICC) were 0.91 for all scored radiographs, 0.84 for baseline radiographs and 0.97 for the radiographic progression rate.

Statistical analysis

Four different treatment strategies were applied to patients included in the EAC depending on the year of inclusion. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or sulphasalazine if they had persistent active disease (delayed treatment).[2] From 1996 to 1998 patients were promptly treated with either chloroquine or sulphasalazine (early treatment).[2,3] From 1998 to 2002 patients were promptly treated with either sulphasalazine or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either sulphasalazine or methotrexate combined with treatment adjustments based on disease activity (early and disease activity based treatment).

To take advantage of the prospective character of the EAC, consisting of repeated measurements, and to avoid multiple testing by performing statistical tests for each time point, a linear mixed model for longitudinal data was used, with the log transformed sharp score as response variable, to compare the radiological progression between genotype groups. We explored different correlation structures between the repeated measurements, and based on the Akaike's information criterion, an autoregressive correlation structure with heterogeneous variances was chosen. This model takes missing observations into account, assuming that the missing is at random. Differences in progression rates between the different genotypes were tested by considering the significance of the interaction between genotype and time with time as linear covariate. The year of inclusion into the study was entered into the model to correct for possible confounding effects. Inclusion period is a proxy for treatment modalities, because treatment strategies improved over time and an influence of the treatment strategy on the progression of radiographic joint damage was observed previously.[2] The interaction between treatment strategy (i.e. inclusion year) and time was significant in all five analyses of the present study ($p<0.05$).

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Supplementary table 1. Comparison of the LD-parameters obtained from HapMap (CEU population in rel. 24 Phase II Nov 08) and the Leiden dataset

		HapMap CEU		Leiden Dataset	
SNP1	SNP2	D'	r^2	D'	r^2
rs1878658	rs675520	1.0	0.112	1.0	0.155
rs1878658	rs9376293	1.0	0.274	1.0	0.263
rs1878658	rs10499194	1.0	0.623	0.98	0.5
rs1878658	rs6920220	1.0	0.028	0.929	0.04
rs675520	rs9376293	0.931	0.356	0.893	0.482
rs675520	rs10499194	1.0	0.191	0.98	0.289
rs675520	rs6920220	1.0	0.209	0.987	0.289
rs9376293	rs10499194	1.0	0.441	0.982	0.478
rs9376293	rs6920220	1.0	0.102	0.985	0.172
rs10499194	rs6920220	1.0	0.045	0.988	0.087

Supplementary table 2. Median Sharp van der Heijde scores (M) and interquartile ranges (IQR; 25 - 75% percentiles) per genotype for ACPA- RA patients (# = number of patients). Genotypes were combined for rs1878658 (G), rs10499194 (T) and rs6920220 (A), as the frequency of patients homozygous for the respective minor allele was ≤5% for these SNPs.

	Year																	
	0			1			2			3			4			5		
ACPA-	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#
rs1878658																		
AA	4	2 - 10	103	6	3 - 12	95	8	3 - 18	79	9	5 - 17.5	69	9	3 - 19.5	57	10	5 - 22	47
AG/GG	5.5	1 - 12	30	7	4 - 15	25	11.5	4 - 21.5	28	11	4 - 21	23	14	4 - 24	23	11	4 - 27	18
rs675520																		
AA	4	2 - 7	28	5.5	1.5 - 16	26	8	5 - 21	21	8.5	4 - 14	20	8	2.5 - 19	16	9.5	5.5 - 19.5	16
AG	5	1 - 10	71	6	3 - 12	64	7.5	3 - 14.5	56	8	5 - 19	49	10	3 - 20	42	10	4.5 - 20.5	33
GG	5.5	1 - 11	36	8	2 - 21	31	9.5	4 - 21.5	32	12	7 - 22.5	24	14	5 - 26	23	14.5	6 - 31.5	16
AG/GG	5	1 - 10	107	7	3 - 12	95	8	3 - 16.5	88	10	5 - 20.5	73	11	4.5 - 23.5	65	11	5 - 27	49
rs9376293																		
CC	4.5	0.5 - 11	22	5	1 - 10	19	9	1 - 12	19	12	5 - 19.5	17	12	2.5 - 20	13	9	3.5 - 44.5	8
CT	5	2 - 11	69	7	4 - 17.5	61	8	4 - 20.5	57	11	5 - 21.5	44	12	5 - 25	43	12.5	6 - 27	34
TT	4	2 - 7	43	5	2 - 10	41	6.5	3 - 18.5	32	7	3 - 11	31	6	3 - 10.5	24	9	4 - 14	23
CC/CT	5	1 - 11	91	7	4 - 15	80	9	3.5 - 17.5	76	11	5 - 21.5	61	12	5 - 23.5	56	12	5 - 27	42
rs10499194																		
CC	4	1.5 - 7	76	5.5	2.5 - 11.5	70	7	3 - 16	56	8.5	5 - 16.5	52	8	3 - 17.5	44	9.5	5.5 - 20.5	36
CT/TT	5	1 - 12	53	8	3 - 17.5	46	10	3.5 - 21.5	49	11	4.5 - 22	38	12.5	4.5 - 24.5	36	12	4 - 27	28
rs6920220																		
AA/AG	5	2 - 8.5	45	6	3 - 12	39	6	3 - 21	31	6	3 - 14	29	6	3 - 12	23	9.5	2.5 - 17.5	18
GG	4.5	1 - 10	88	7	2.5 - 13.5	81	8.5	4 - 18	76	10.5	6 - 20.5	62	12	5 - 22.5	57	11	5 - 27	46

CHAPTER 7

Association of a single-nucleotide polymorphism in *CD40* with the rate of joint destruction in rheumatoid arthritis

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ABSTRACT

Objective

The severity of joint destruction in rheumatoid arthritis (RA) is highly variable between patients and influenced by genetic factors. Genome-wide association studies (GWAs) have boosted the field of the genetics of susceptibility to RA enormously, but risk loci for severity of RA remain poorly defined. A recent meta-analysis of GWAs identified 6 genetic regions for susceptibility to autoantibody-positive RA, i.e. *CD40, KIF5A-PIP4K2C, CDK6, CCL21, PRKCQ* and *MMEL1-TNFRSF14*. We have investigated whether these newly described genetic regions associate with the rate of joint destruction.

Methods

RA patients enrolled in the Leiden Early Arthritis Clinic were studied (n=563). Yearly radiographs were scored using the Sharp-van der Heijde method (median follow-up 5 years, maximal follow-up 9 years). The rate of joint destruction between genotype groups was compared using a linear mixed model correcting for age, gender and treatment-strategies. 393 ACPA-positive RA patients included in the NARAC with radiographic data were used for replication.

Results

The TT and CC/CG genotypes of two SNPs, rs4810485 *(CD40)* and rs42041 *(CDK6)* respectively, were associated with a higher rate of joint destruction in ACPA-positive RA (p=0.003 and 0.012), of which rs4810485 was significant after Bonferroni correction for multiple testing. The association of the *CD40* minor allele with radiographic progression rate was replicated in the NARAC cohort (p=0.021).

Conclusion

A polymorphism in the *CD40* locus is associated with the rate of joint destruction in ACPA-positive RA and provides one of the first non-HLA-related genetic severity factors that is replicated.

INTRODUCTION

Rheumatoid arthritis (RA) is characterized by inflammatory arthritis and localized destruction of bone and cartilage. The severity of joint destruction is highly variable between patients and, according to twin studies, substantially influenced by genetic factors.[1] Nevertheless, the precise contribution of genetic factors still has to be determined. To date only a small number of genetic risk factors has been identified, and apart from HLA, none of these factors have been convincingly replicated.

In contrast, the genetics of susceptibility to RA has been boosted considerably, largely due to genome-wide association studies. In addition to the HLA-DRB1 shared epitope alleles, several new susceptibility factors, *PTPN22*, *TRAF1-C5*, *OLIG3-TNFAIP3* and *STAT4*, have been identified and were independently replicated. Intriguingly, for many of these genetic risk factors the associations are confined to anti-citrullinated protein antibodies (ACPA)-positive RA patients. Whether genetic factors also differently affect the severity of joint destruction in ACPA-positive and ACPA-negative RA remains unknown. Nonetheless, compelling evidence demonstrates that ACPA-positive RA patients have a more destructive disease course compared to ACPA-negative patients.

A recent meta-analysis on two genome-wide association studies identified six new risk loci (rs4810485 (*CD40*), rs1678542 (*KIF5A-PIP4K2C*), rs42041 (*CDK6*), rs2812378 (*CCL21*), rs4750316 (*PRKCQ*) and rs3890745 (*MMEL1-TNFRSF14*)) as susceptibility factors for autoantibody-positive RA.[2] The present study aimed to investigate the association between these single nucleotide polymorphisms (SNPs) and the rate of radiological joint destruction in RA, and ACPA+ RA in particular, using a large longitudinal cohort. A cohort of ACPA-positive RA patients was used for replication. This study shows that a genetic variant in the *CD40* gene associates with the rate of joint destruction in ACPA-positive RA.

PATIENTS AND METHODS

Patients

Five hundred sixty three RA patients, consecutively included in the Leiden Early Arthritis Cohort (EAC) between 1993 and 2006 with both DNA and radiographs available were studied. The RA patients fulfilled the 1987 ACR-criteria. Follow-up visits were performed yearly. Treatment strategies changed in time and differed for different inclusion periods (before 1996, 1996-1998, 1999-2001, after 2001) (see reference 3 for detailed description of the EAC). Anti-CCP2 antibodies were measured using stored baseline serum samples (Immunoscan RA Mark 2; Euro-Diagnostica, The Netherlands).

Replication cohort

393 ACPA-positive RA patients that were included in the North American Rheumatoid Arthritis Consortium (NARAC) that had hand radiographs available were studied. As the radiographs were taken at different disease durations, the estimated radiological progression per year was determined by dividing the total Sharp-van der Heijde score of the hands by the disease duration at the time of the radiograph.

SNP genotyping

The six recently identified risk loci[2] were genotyped in the 563 RA patients from the Leiden EAC using allele-specific kinetic PCR as previously described.[4] The data were hand-curated without knowledge of clinical characteristics before statistical analysis with a 98% genotyping success rate; previous analyses suggest a genotyping accuracy of >99%. For the *MMEL1-TNFRSF14* locus, a perfect proxy of rs3890745 (as reported[2]) was used (rs6684865, r^2=1).

In the NARAC genotyping was performed using the Illumina Hapmap500 BeadChip, as described.[5] Rs4810485 was not typed in the whole genome study, but a perfect proxy for this variant was genotyped (rs1569723, r^2=1). For *CDK6*, neither rs42041 nor a perfect proxy were genotyped and therefore the data on rs42041 was imputed as described.[2]

Radiographs

In the EAC, radiographs of hands and feet, taken on consecutive years, were scored according to the Sharp-van der Heijde method.[6] To encompass a reliable sample size, radiographic follow-up data were restricted to a maximum of 9 years with a median of 5 years. All radiographs were scored by one experienced scorer who was blinded with respect to clinical and genetic data. 499 radiographs were rescored (149 baseline radiographs and 350 radiographs during follow-up from 60 randomly selected RA patients). Intraclass correlation coefficients (ICC) were 0.91 for all radiographs, 0.84 for baseline radiographs and 0.97 for the radiographic progression rate. In the NARAC the radiographs were scored by one reader blinded to clinical or genetic data. 25% of the radiographs were re-scored, the ICC was 0.99.

Statistical analysis

Analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). As radiographic data were not normally distributed, the raw data on the Sharp-van der Heijde scores are presented using medians and were log-transformed in preparation for analysis. In the EAC, a linear model for longitudinal data was used to compare progression rates between groups. Age, gender, inclusion period (a proxy for treatment strategy) and their interactions with time were entered in the model to correct for possible confounding effects (see below). As six SNPs were evaluated, a Bonferroni correction for multiple testing was applied; the p-value for significance was set at $p<0.008$. Only the SNPs that were clearly related to the progression rate in the EAC were analyzed in the replication cohort. In the NARAC, the estimated radiological progression per

year was compared with the Kruskal-Wallis test. No corrections were made for age, gender or treatment in this cohort.

To take advantage of the prospective character of the data of the EAC, consisting of repeated measurements, and to avoid multiple testing by performing statistical tests for each time point, a linear model for longitudinal data was used, with the log transformed Sharp-score as response variable, to compare the radiological progression rates between genotype groups. Different correlation structures between the repeated measurements were explored, and based on the Akaike's information criterion, an autoregressive correlation structure with heterogeneous variances was chosen. Due to the study design (an inception cohort) not all patients achieved a similar duration of follow-up. The model takes missing observations into account, assuming that the missing is at random. Differences in progression rates between the different genotypes were tested by considering the significance of the interaction between genotype and time with time as linear covariate. Age, gender and inclusion period (before 1996, 1996-1998, 1999-2001, after 2001) and their interactions with time were entered in the model to correct for possible confounding effects. In order to prevent overfitting of the data no corrections were applied for other variables. Inclusion period is a proxy for treatment modalities, because treatment strategies improved over time and an influence of the treatment strategy on the progression of radiographic joint damage was observed previously, as well as in the present study. The following treatment strategies were applied in the subsequent inclusion periods.. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or sulfasalazin if they had persistent active disease (delayed treatment). From 1996 to 1998 RA patients were promptly treated with either chloroquine or sulfasalazin (early treatment).[3] From 1998 to 2002 patients were promptly treated with either sulfasalazin or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either sulfasalazin or methotrexate combined with treatment adjustments based on the disease activity (early and disease activity based treatment).

RESULTS

Baseline characteristics of the RA patients are shown in Table 1. In the EAC, the minor allele frequencies were 0.242, 0.340, 0.267, 0.366, 0.204 and 0.307 for rs4810485, rs1678542, rs42041, rs2812378, rs4750316 and rs6684865 respectively and in agreement with previous results.[2] The raw data on the Sharp-van der Heijde scores for the three genotypes at each SNP are depicted in Figure 1. To study the influence of the SNPs on the rate of joint destruction, a linear mixed model analysis was performed for each SNP. For rs4810485 (*CD40*) the GG and GT genotypes showed comparable radiographic scores, therefore the genotype data were combined and carriership-analysis was performed. Similarly, the CC and CG genotypes of rs42041 (*CDK6*) were pooled. In the total group of RA patients an association was observed for rs42041 (*CDK6*) (p=0.033). For the other SNPs no significant association with the radiological progression over time was detected (p=0.268, 0.369, 0.679, 0.583 and 0.451 for rs4810485, rs1678542, rs2812378, rs4750316

Table 1. Patient characteristics at baseline

Patient Characteristics EAC	N=563
Age at inclusion (yrs), mean (SD)	56.0 (15.6)
Female, N (%)	394 (70.0)
Symptom duration at inclusion (months), mean (SD)	6.7 (10.5)
Swollen Joint Count, mean (SD)	5.72 (3.3)
Ritchie score, mean (SD)	10.3 (7.8)
ACPA-positive, N (%)†	250 (55.9)
IgM-RF-positive, N (%)†	322 (58.4)
HLA-DRB1 Shared Epitope +, N (%)†	339 (67.1)
CRP (mg/l), mean (SD)†	29.4 (34.2)
ESR (mm/h), mean (SD)†	39.5 (27.5)
HAQ, mean (SD)	1.1 (0.7)
Total Sharp-score, median (IQR)	5 (2-11)
Patient Characteristics NARAC	N=393
Age at disease onset	40.8 (11.9)
Female, N (%)	286 (72.8)
ACPA-positive, (%)§	100%
HLA-DRB1 Shared Epitope +, N (%)§	100%

†Data on ACPA-, RF- and HLA DRB1 SE-status and CRP and ESR-levels were available in the EAC in 447, 551, 441, 520 and 544 out of 563 genotyped patients respectively. §Data on ACPA- and HLA-DRB1 SE-status was available for all of the 393 genotyped patients

and rs6684865 respectively). Because the genetic regions studied are thus far observed to be susceptibility factors only for autoantibody-positive RA patients, analyses were repeated in the ACPA-positive subgroup. Here, two polymorphisms, rs4810485 (*CD40*) and rs42041 (*CDK6*), affected the rate of joint destruction (Figure 2). For rs4810485, the G-allele was associated with a lower progression rate (GG/GT vs. TT, p=0.003). Back transforming the regression coefficient of the genotype in the model to the original scale yielded a 1.12 (95% CI 1.04-1.21) times larger increase in Sharp-score per year for carrying the risk genotype. For rs42041, the C-allele was associated with a higher rate of joint destruction (CC/CG vs. GG, p=0.012). For carriership of the C-allele a 1.09 (95% CI 1.02-1.16) larger yearly increase in Sharp-score was observed. Only rs4810485 was statistically significant after correction for multiple testing. The interaction between inclusion period and time was significant in all six analyses (p<0.001), demonstrating the effect of inclusion period on the radiological progression rate. Gender and age were not independently associated with progression.

To find replication, the effect of *CD40* and *CDK6* on radiological progression was analysed in 393 ACPA-positive RA patients from the NARAC. Using a perfect proxy for rs4810485, the genotype associated with severity in the EAC also revealed a higher estimated radiological progression per year in the NARAC: 3.40 Sharp-units/year (n=23) vs. 2.83 and 1.83 Sharp-units/year

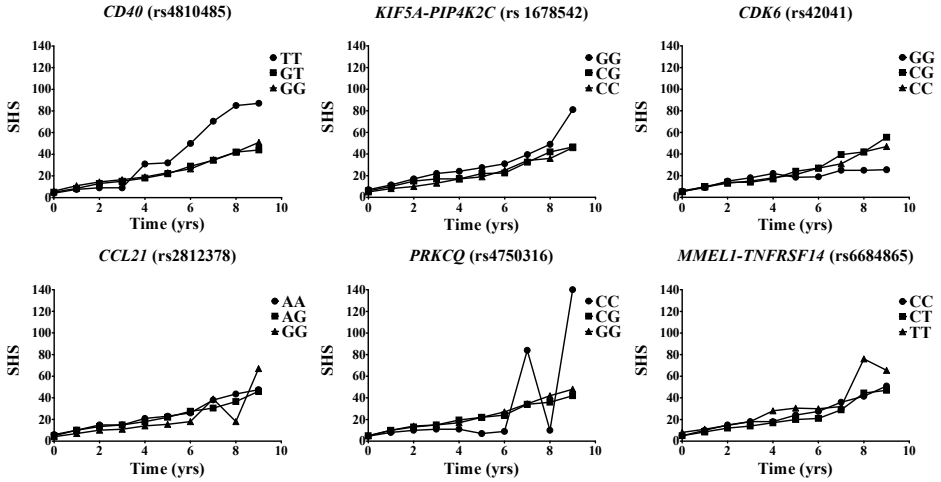

Figure 1. Median Sharp-van der Heijde scores for the different SNPs per genotype in all RA patients. Overview of the raw Sharp-van der Heijde scores, expressed as medians, of all 6 SNPs per genotype for the total patient population (n=563). The risk-alleles predisposing to RA in the study of Raychaudhuri et al[2] were the G-, C-, G-, G-, G- and T-allele for the rs4810485, rs1678542, rs42041, rs2812378, rs4750316 and rs6684865 SNPs respectively. The number of available radiographs varied per time-point and declined to 466 after 1 year of follow-up, 426, 357, 299 and 269 after 2 till 5 years of follow-up and 206, 154, 116 and 84 radiographs after 6, 7, 8 and 9 years of follow-up respectively. The number of patients in the different genotype groups were respectively: GG:280, GT:198, TT:22 (rs4810485)*; CC:247, CG:248, GG:67 (rs1678542); CC:305, CG:215, GG:43 (rs42041); AA:217, AG:279, GG:66 (rs2812378); CC:23, CG:183, GG:355 (rs4750316); CC:166, CT:170, TT:26 (rs6684865)*. *Due to technical difficulties genotyping was not successful in 63 and 201 of cases for rs4810485 and rs6684865 respectively. SHS: Sharp-van der Heijde score

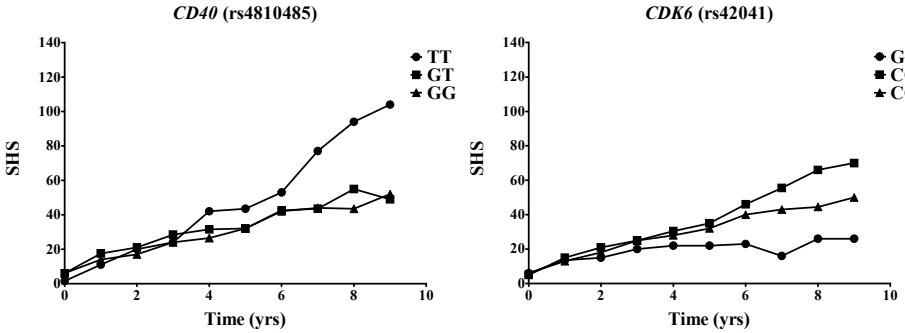

Figure 2. Median Sharp-van der Heijde scores for rs4810485 and rs42041 in ACPA-positive RA. Overview of the raw Sharp-van der Heijde scores, expressed as medians, in ACPA-positive RA (n=250). The G-allele was the risk-allele predisposing to RA in the study of Raychaudhuri et al[2] for both the rs4810485 and rs42041 SNPs. The number of patients in the different genotype groups were for rs4810485*: GG: 128, GT: 88, TT: 11 and for rs42041: CC: 131, CG: 101, GG: 18. *Genotype data for rs4810485 was not available in 23 cases. SHS: Sharp-van der Heijde score

(n=122 and 248, p=0.021). Using imputed data for rs42041 no significant differences between the three genotypes were observed (2.76, 2.38 and 2.07 Sharp-units/year, n=32, 163 and 188 respectively, p=0.327). The total number of patients available for analysis of rs42041 was 383; genotyping data were missing in 10 cases.

DISCUSSION

Although several clinical and serological risk factors for RA severity are known, thus far the inter-individual variance in joint destruction is insufficiently explained and genetic factors are scarcely investigated. A better comprehension of the factors that mediate joint damage in RA may lead to the development targeted therapies or may contribute to prediction of the disease outcome in individual RA patients. Most recently, six new loci were described to predispose to autoantibody-positive RA.[2] Although susceptibility factors do not necessarily affect disease progression, this study investigated whether these six SNPs are also risk factors for a severe course of RA, measured by the rate of joint damage. The present data suggest that two SNPs, rs4810485 (*CD40*) and rs42041 (*CDK6*), influence the rate of joint destruction in ACPA-positive RA. Of these, only rs4810485 was significantly associated after correction for multiple testing and was replicated in an independent cohort of ACPA-positive RA patients. As such, *CD40* is the first non-HLA-related genetic risk factor for RA severity that is independently replicated.

A recent study[2] reported a common variant at the *CD40* locus (the minor T-allele) to be protective for the development of RA. Surprisingly, here the minor T-allele associates with a higher rate of joint destruction in two cohorts. This finding is counter-intuitive, if one assumes that genetic variants associating with susceptibility also associate with severity. Although our findings were observed in two independent cohorts, and thus replicated, a type I error cannot be ruled out. The disease associated (common) allele marks a haplotype of *CD40* that contains a polymorphism in the upstream Kozak sequence that results in increased surface expression on B cells.[7] To our knowledge, the effect of this haplotype on CD40 surface expression in synovial fibroblasts has not been directly studied. However, CD40 expression is increased on synoviocytes in RA and triggering of CD40 in synovial fibroblasts is associated with production of proinflammatory cytokines and osteoclastogenesis.[8,9] It is likely that the biological pathways underlying susceptibility and severity are distinct with respect to CD40 triggering. This would provide an explanation for the finding that the minor T-allele has a protective effect in susceptibility studies but associates with a more severe disease course. Clearly it is essential to perform further studies on the mechanisms by which *CD40* polymorphisms associate with erosive outcome in RA.

A second SNP tended to associate with the rate of joint damage in RA in the EAC, rs42041. Absence of replication in the NARAC indicates that the observed association with the progression rate in the EAC cannot be interpreted. Nonetheless, it will be interesting to see the results on other studies analyzing *CDK6* and RA severity. Thus, at present, of the two SNPs that tended to show an association with the rate of joint destruction, only the genetic variant in *CD40* is statisti-

cally significant after correction for multiple testing and is replicated and is therefore identified as a severity factor for RA.

The other four studied SNPs in the loci encoding for *KIF5A-PIP4K2C, CCL21, PRKCQ* and *MMEL1-TNFRSF14* were not observed to associate with the severity of joint destruction. Therefore, these polymorphisms appear to be genetic risk-factors that are primarily associated with RA susceptibility. Indeed, all of these SNPs were recently replicated as true susceptible loci in RA patients of European ancestry.[10]

The prospective nature of the data of the EAC strengthens the impact of the findings because higher radiological scores for risk genotypes were present at subsequent time points; as such the present data set is advantageous in comparison to studies that assessed cross-sectional radiological data. The fact that a large number of patients with a long follow-up of up to 9 years were included for analysis is clearly an advantage, but also has a limitation. Inherent to the design of an inception cohort, not all patients had achieved maximum follow-up, so the number of missing data that the mixed-model had to take into account increased with longer follow-up. Small numbers of radiographs available at the latest time points are also the most likely explanation for the observed "bump" at the 8 year time point for the genotypes GG, CC and TT of the SNPs rs2812378, rs4750316 and rs6684865 respectively (Figure 1).

Evaluation of the effect of genetic factors on the rate of joint destruction during the disease course inevitably implies that other factors that affect the disease course should be taken into consideration as well. Analyses for all six SNPs revealed that inclusion period, a proxy for treatment strategy, was significantly associated with the rate of joint damage, which is in line with previous results from the EAC.[11] The analyses on *CD40* and *CDK6* showed that these SNPs were associated with joint damage, independent from treatment strategy. Nevertheless, corrections for treatment strategy were made on group-level and thus were an approximation for the real effect of treatment on the rate of joint destruction for individual RA patients.

In conclusion, a polymorphism in the *CD40* locus shows a significant association with the rate of joint destruction in ACPA-positive RA, a finding that is replicated in an independent cohort. Although further studies are needed to identify the causal variant, the data presented provide a foundation for further investigations of the role of CD40 in joint destruction in RA.

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CHAPTER 8

The *PTPN22* susceptibility risk variant is not associated with the rate of joint destruction in anti-citrullinated protein antibody-positive rheumatoid arthritis

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A missense Single Nucleotide Polymorphism (SNP) in the protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) gene, that encodes an negative regulator of T-cell activation, is an important genetic risk factor for rheumatoid arthritis (RA) susceptibility.[1] The association of *PTPN22* susceptibility risk allele and severity of joint destruction is unclear due to contradictory observations.[2-6] To determine an individual patient's rate of joint destruction accurately, it is required that radiological measurements are collected via standard procedures, scored quantitatively and sensitively and are repeated in time. Consequently, differences in used measurement and analysis methods may contribute to the occurrence of contrasting findings. Second, although the effect of *PTPN22* on RA susceptibility is confined to the ACPA-positive group,[2,6] most studies on *PTPN22* and joint destruction did not analyze the ACPA+ subset.[2-5] The present study studied the effect of the *PTPN22* susceptibly risk variant on the rate of joint destruction in two large cohorts of ACPA+ patients, using sensitive methods for measurement and analysis.

The first cohort consisted of 593 RA patients from the Leiden Early Arthritis Clinic (EAC),[7] of whom 55% were ACPA-positive. Radiographs were made at baseline and on consecutive years. The radiographs were scored by one experienced scorer. The intraclass-observer correlation coefficient was 0.91. The progression in Sharp-van der Heijde score (SHS) during 6 years of follow-up was compared between RA patients with and without the risk variant (T-allele) of rs6679677, a perfect proxy for rs2476601/C1858T (r^2=1), using a repeated measurement analysis. Such analysis takes advantage of the longitudinal, repetitive character of the data and does not exclude patients with incomplete follow-up data, avoiding selection bias. In a linear mixed model with radiological score as response variable, the effect of time was assumed to be linear in the interaction terms. *PTPN22* and its interaction with time were entered in the model, to test whether *PTPN22* T/non-T carriers had different radiological scores over time. Age, gender and inclusion period (a proxy for treatment strategy) were entered in the model to correct for possible confounding effects.[8]

The replication cohort consisted of 397 ACPA+ patients North American Rheumatoid Arthritis Consortium (NARAC) with cross-sectional radiological measurements (SHS) and genotypic data of rs2476601. Estimated radiological progression rates per year were compared using the Mann-Whitney test. In this cohort, no corrections were made for age, gender or treatment.

In the first cohort, 69.0% of patients were female and the mean age was 56.4 ± 15.8 years. The genotype frequencies (GG/GT/TT) were 462/120/11 (77.9%/20.2%/1.9%). The presence of the T-allele (TT+TG-genotype) was not associated with a higher rate of radiological joint destruction compared to the absence of this allele (GG-genotype) (p=0.10 and p=0.93 respectively in ACPA-positive and in all patients) (Figure 1). In the second cohort, 72.8% of the patients were female and the mean age was 40.8 ± 12.0 years. The genotype frequencies (CC/CT/TT) were 282/105/10 (71%/26%/3%). Again, no significant difference in estimated radiologic progression per year was found (median 2.11 Sharp units per year in the CC group versus 2.4 Sharp units per year in the TT+TC-group, p=0.22). Exclusion of ten genetic outliers did not change these results.

Using the present EAC data, this study had a power of 0.986 to detect a difference of 2.14 SH-scores with a SD of 4.07 (difference in increase in SHS over 6-years) and an alpha of 0.05; indicating this study was sufficiently powered to prevent false negative findings.

In conclusion, this study shows that *PTPN22,* although it predisposes to ACPA-positive RA, is not associated with RA severity measured by the radiological rate of joint destruction, proving a further indication that the contribution of *PTPN22* to RA is primarily found in setting the balance involved in the emergence of ACPA.

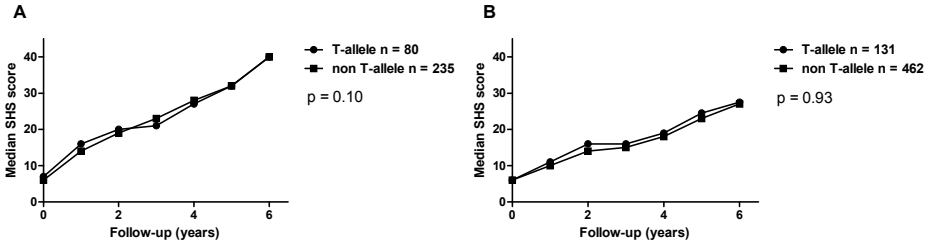

Figure 1. Median Sharp van der Heijde scores during 6 years of follow-up for patients with and without the T-allele of *PTPN22* in ACPA+ RA (A) as well as all RA (B) in the EAC. Three hundred fifteen ACPA-positive patients had radiographs available. The number of radiographs declined from 303 to 267, 251, 212, 185, 169 and 139 respectively from baseline to 6 year follow-up. The available radiographs of the total RA population were in total 593, this declined to 577, 488, 442, 365, 309, 263 and 212 respectively from baseline till 6 year follow-up

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PART IV

Delay in referral and RA severity

CHAPTER 9

Long-term impact of delay in assessment of early arthritis patients

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ABSTRACT

Background

During the last decade rheumatologists have learned to initiate disease-modifying-antirheumatic-drugs (DMARDs) early to improve outcome of rheumatoid arthritis (RA). The effect of delay in referral to rheumatologists on the outcome of RA is scarcely explored. We studied the association between delay in assessment by rheumatologists, rates of joint destruction, and probability of achieving DMARD-free-remission in RA. Patient characteristics associated with the patient and general practitioner (GP)-components of overall delay were assessed.

Methods

1674 early arthritis-patients from the Leiden EAC were studied on patient, GP-, and total delays. Within 598 RA patients, associations between total delay, achievement of sustained DMARD-free remission, and the rate of joint destruction over six years follow-up were determined.

Results

The median patient, GP-, and total delays in early arthritis-patients were 2.4, 8.0 and 13.7 weeks respectively. From all diagnoses, early arthritis patients diagnosed with RA or spondylarthropathy had the longest total delay (18 weeks). 69% of RA patients were assessed in ≥12 weeks; this was associated with a hazard ratio of 1.87 for not achieving DMARD-free remission and a 1.3 times higher rate of joint destruction over six years compared to assessment <12 weeks. Older age, female gender, gradual symptom onset, small joint involvement, lower CRP levels, and autoantibody presence associated with longer total delay.

Conclusion

Only 31% of RA patients were assessed <12 weeks. Assessment <12 weeks is associated with less joint destruction and a higher chance on DMARD-free remission compared to a longer delay in assessment. These results imply that attempts to diminish delay in seeing rheumatologists will improve disease outcome in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic disease, affecting 1% of the population. It is associated with significant morbidity, mortality and cost for the health service and society. The disease is characterized by inflammation of the synovium, most frequently in the small joints of hands and feet; this inflammatory process frequently leads to loss of cartilage and bone erosions. The level of joint destruction is correlated with the severity of inflammation.[1,2] At present, potent Disease Modifying Antirheumatic Drugs (DMARDs) and biological agents are available to treat RA synovitis. It has been unequivocally demonstrated that early initiation of aggressive treatment schedules results in less joint damage and disability.[3-6] This has led to the concept of the 'window of opportunity'.[7] Indeed it has been demonstrated that initiation of treatment within 12 weeks after disease onset results in lower levels of joint destruction[8] and increases the chance of achieving remission,[9] which is increasingly regarded as the targeted outcome in therapeutic trials. Many studies focused on the importance of diminishing delay between the diagnosis of RA and treatment initiation. However, shortening the time period between first symptoms and first visit to a rheumatologist might be equally important. Thus far the effect of delayed assessment by rheumatologists on disease outcome has scarcely been investigated.

We aimed to assess the association between delay in assessment and disease outcome in RA, measured by the rate of joint destruction and the chance of achieving sustained DMARD-free remission. Second, we also aimed to determine the patient characteristics associated with longer patient and GP-delay. Knowledge of these factors is of utmost importance. Rheumatologists nowadays are aware of the need to treat early. This implies that to further improve the outcome of RA, strategies should be put in place to ensure that delays in assessment are as short as possible. Understanding factors that associate with delayed assessment is the first step required to achieve this.

PATIENTS AND METHODS

Patients

All patients come from the Leiden Early Arthritis Clinic (EAC) cohort, a large inception cohort that enrolled all consecutive patients between 1993 and 2006.[10] This clinic is the only referral center in a health care region of about 300,000 inhabitants. Patients were referred by their general practitioners (GPs) when arthritis was suspected and GPs were encouraged to refer as soon as possible. Inclusion took place when synovitis was confirmed by physical examination and symptom duration was less than 2 years. At baseline, patients were asked about their joint symptoms and subjected to a physical examination, which included a 66 swollen and 68 tender joint count (Ritchie score). Blood samples were taken for routine diagnostic laboratory screening (including C-reactive protein (CRP) and IgM-rheumatoid factor (RF)) and stored to determine other autoantibodies (anti-CCP2) at a later time. Follow-up visits were performed on a yearly

basis and included radiographs of hands and feet.[10] Written informed consent was obtained from all participants. The study was approved by the local Medical Ethical Committee.

Of all 1881 patients included in the EAC cohort, information on the dates of symptom onset was available for 1674 patients. There were no significant differences between baseline patient characteristics of patients with and without information on this date, apart from slightly lower titers of acute phase reactants in the group with missing data (data not shown). Among the 1674 patients who had information on the date of symptom onset available, 598 patients (35.7%) were diagnosed with RA according to the 1987 ACR criteria within the first year of follow-up and had radiographs available. These patients were consecutively included between 1993 and 2006. Treatment strategies for RA changed over time and became more aggressive in subsequent inclusion periods (1993-1996, 1996-1998 and 1999-2006).[10] Patients included before 1996 were treated initially with analgesics and subsequently with chloroquine or salazopyrin if they had persistent active disease (delayed treatment). Between 1996 and 1998 RA patients were promptly treated with either chloroquine or salazopyrin, and from 1999 onward patients were promptly treated with either salazopyrin or methotrexate.

Delay

We studied delay at 2 levels. Level 1 related to the delay from the onset of symptoms to a patient being seen by their GP. This delay is a composite of the delay on the part of the patient in seeking an appointment with the GP and the time the patient has to wait to see the GP once they have approached the GP for an appointment. In practice, the Dutch healthcare system is such that the second component of this is almost always very short and for simplicity we have referred to level 1 delay as "patient delay". Level 2 delay related to the delay from when the patient first saw their GP to when they were seen in the Leiden Early Arthritis Clinic. This delay is also a composite; in this case of the time it takes a GP to decide to make a referral and the time it takes for the rheumatologist to see the patient once the referral is made. The average wait for a patient to be seen in the Leiden EAC, once a referral has been made, is short (~2 weeks) and for simplicity we have referred to level 2 delay as "GP-delay". The total delay was calculated as the sum of both patient and GP-delay. The duration of total delay was known for 1674 early arthritis patients. Data on the first visit to a GP was available for ~1100 early arthritis patients. There were no significant differences between characteristics of patients with and without information on the date of visiting the GP (data not shown). Analysis of associations between patient characteristics and delay were carried out for patient delay, GP-delay, and total delay. For all other analyses, the total delay was used. Since the literature indicates that the time period known as 'the window of opportunity' is about 12 weeks, the total delay was divided into two categories: <12 weeks and ≥12 weeks.[7-9]

Radiographs

Radiographs of hands and feet of 598 RA patients were scored according to the Sharp-van der Heijde method.[11] Due to the study design (an inception cohort) not all patients had an equal duration of follow-up (median 4 years, IQR 2-6). Radiographic follow-up data were restricted to a maximum of 6 years because of increasing frequency of missing radiographs later on. All radiographs were scored by one experienced scorer (MPMvdL) who was blinded with respect to clinical and treatment data. 499 radiographs were rescored (149 baseline radiographs and 350 radiographs during follow-up from 60 randomly selected RA patients). Intraclass-observer correlation coefficients (ICC) were 0.91 for all radiographs, 0.84 for baseline radiographs, and 0.97 for the radiographic progression rate.

Sustained DMARD-free remission in RA

Remission was defined in its most stringent form as the persistent absence of synovitis for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheumatologist.[12] As such, this definition approaches cure of the disease. The remission status could be reliably ascertained in 557 out of 598 RA patients. 72 Patients (12.9%) achieved sustained DMARD-free remission after a median follow-up of 3.33 years (IQR 2.02-5.48). Most patients who achieved remission had a synovitis-free follow-up longer than the minimum requirement of one year; the median time of observation after achieving sustained DMARD-free remission was two-and-a-half years.

Statistical analysis

The duration of patient delay and GP-delay within a patient were compared using the Wilcoxon signed ranks test.

The association between delay and the rate of joint destruction during follow-up after the visit to a rheumatologist was assessed in 598 RA patients using repeated measurement analysis on log-transformed radiological data of subsequent yearly measurements. Log transformation was performed because of skewness of radiological data. Visit number and delay group were entered as categorical variables. Adjustments were applied for age, gender, and inclusion period (a proxy for treatment strategy) and their interaction with time as described before,[13] since these factors are known to influence the rate of joint destruction. Difference in the rate of joint destruction between the delay groups was assessed by testing the interaction between time and delay group. The association between delay and disease progression was also analyzed with the onset of symptoms as a starting point. This was done with a repeated measurement analysis with a random person and time effect, where the fixed effect of time was modeled with linear spline functions with knots at each year.

Analysis of sustained DMARD-free remission was performed by comparing Kaplan-Meier curves and by Cox regression analysis, taking into account the differences in follow-up times among patients. For patients who achieved remission, the dependent variable was "time-to-

event", indicating the time until reaching remission. For non-remission patients the time to last follow-up was used. Again two different starting points were considered: time from the onset of symptoms and time from the first visit to a rheumatologist. Cox regression for left truncated data was used for the analysis with time from onset of symptoms to account for the fact that remission status was only observed after the first visit to a rheumatologist.

Univariate analyses of baseline patient characteristics associating with delay in early arthritis patients were performed using Mann-Whitney U and Kruskal-Wallis tests as delay data were not normally distributed. In order to identify baseline characteristics that independently associated with delay, variables that associated with delay in univariate analyses ($p<0.05$) were entered in a multivariate regression analysis with backward selection method. For these analyses delay data were log-transformed. To prevent exclusion of patients with missing data from the multivariate model, multiple imputations were performed (SPSS 17.0). The complete set of data was used to generate 10 imputations that were subsequently applied to the multivariate analysis.

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and R (http://www.R-project.org) were used. P-values <0.05 were considered significant. All reported p-values are two-sided.

RESULTS

Duration of delay in assessment

Baseline characteristics of all early arthritis patients and the patients that were diagnosed with RA are presented in Table 1.

In all early arthritis patients the median total delay was 13.7 (IQR: 5.7-28.5) weeks, the GP-delay 8.0 (IQR: 2.7-18.4) weeks, and the patient delay 2.4 (IQR: 0.7-7.4) weeks. The GP-delay was significantly longer (median 8.0 weeks) than the patient delay (median 2.4 weeks) ($p<0.0001$). The total delay in the subgroup of early arthritis patients who developed RA within the first year of follow-up was 18.4 weeks (median, IQR: 10.4-35.0). Also here, the GP-delay was significantly longer than the patient delay (median 11.8 (IQR: 5.2-22.9) vs. 3.3 (IQR: 1.0-8.9) weeks; $p<0.0001$). The applied treatment strategies for the RA patients differed for three inclusion periods; the median total delays for patients in these inclusion periods were 22.1 weeks for 1993-1996, 18.3 weeks for 1996-1998, and 18.3 weeks for 1999-2006 ($p=0.38$). From all RA patients, only 186 patients (31.1%) were assessed within 12 weeks of symptom onset.

Delay and outcome of RA

Within the 598 patients diagnosed with RA, we investigated whether the degree of delay in assessment has an effect on the disease outcome, measured by the progression in Sharp-van der Heijde score over a six year period of followup and the achievement of sustained DMARD-free remission. Those RA patients who saw a rheumatologist within 12 weeks after symptom onset had a lower rate of progression in Sharp-van der Heijde score (Figure 1A) than those with a delay of ≥12 weeks. Repeated measurement analysis comparing patient groups with delays of

Table 1. Baseline characteristics of all early arthritis patients and the subset of early arthritis patients that were diagnosed with RA

Characteristics	Early arthritis patients (n=1674)	RA (n=598)
Female, n (%)	989 (59.1)	405 (67.7)
Age at inclusion (yrs), mean (SD)	51.7 (17.5)	56.8 (15.8)
SJC, mean (SD)	7.1 (6.4)	9.2 (7.0)
Ritchie score, mean (SD)	7.2 (5.6)	9.2 (6.0)
Anti-CCP2-positive, n (%)	391 (28.5)[§]	309 (53.3)[§]
IgM-RF-positive, n (%)	480 (29.2)[‡]	343 (58.0)[‡]
CRP (mg/l), mean (SD)	28.9 (38.8)	31.0 (35.3)

SJC: 66-swollen joint count; Ritchie score: 68-tender joint count; CRP: C-reactive protein; IgM-RF: Rheumatoid factor. [§]Data on anti-CCP2 status was available for 1373/1674 early arthritis patients and 580/598 RA patients. [‡]Data on IgM-RF was available for 1645/1674 and 591/598 patients respectively

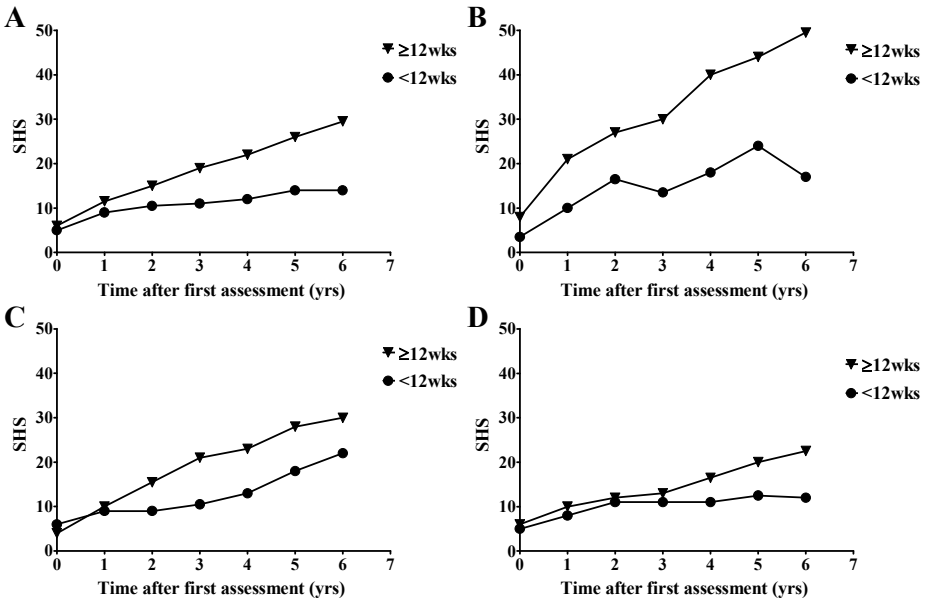

Figure 1. The rate of joint destruction during 6 years of follow-up after first assessment by a rheumatologist for RA patients in different delay categories (A), and separated by treatment strategy after inclusion (B-D). Because of a non-normal distribution of radiological data median Sharp van der Heijde scores are presented. Figure 1A presents data on the total RA group and in Figure 1B-D data were separated for different treatment strategies which became more aggressive over time. The applied treatment strategies were (B) initial treatment with analgesics and subsequently with chloroquine or salazopyrin if they had persistent active disease (delayed treatment), (C) prompt treatment with either chloroquine or salazopyrin, and (D) prompt treatment with either salazopyrin or methotrexate. SHS: Sharp-van der Heijde Score; Time after first assessment (yrs): follow-up time in years after the first visit to a rheumatologist

<12 weeks and ≥12 weeks showed that the difference in progression rate was statistically significant (p=0.001). Because of skewness of the data, radiological data were log transformed before analysis; back transforming the regression coefficient showed that over a period of six years after the first visit to the rheumatologist, patients with a delay ≥12 weeks had a 1.34 fold larger rate of progression in Sharp-van der Heijde score than patients with a delay <12 weeks. In this analysis adjustments were made for age, gender, and the different treatment periods. Plotting the observed median radiological scores over time for the different treatment periods separately (Figure 1B-D), illustrated that RA patients assessed within 12 weeks of symptom onset had lower progression rate, irrespective of the treatment period. Thus although the increase in aggressiveness of treatment after assessment reduced the overall level of Sharp-van der Heijde scores, this did not diminish the effect of delay in referral on progression in Sharp-van der Heijde scores.

The lower progression rate in the patients with a short delay (<12 weeks) could have been due to the fact that these patients presented in an earlier phase of the disease course, with concomitantly less severe joint damage. To investigate whether this explained the observed difference, a second analysis of the progression in Sharp-van der Heijde score was performed while taking into account the symptom duration before the first radiograph, i.e. before presentation. Thus, the follow-up time for all patients now commenced at the (self-reported) first date of symptoms. In this analysis, patients with a delay <12 weeks had a significantly lower progression rate during six years after the onset of the first symptoms, compared to patients with a delay ≥12 weeks (p<0.001 after adjustment for age, gender, and treatment period).

Reports in literature suggest that anti-CCP positive and anti-CCP negative RA are two subsets of RA with differences in the underlying pathophysiological mechanisms and disease course.[14,15] To explore whether the effect of delay was different in anti-CCP positive and negative RA, stratified analyses were performed. Although stratification resulted in reduced power, a statistically significant association of a delay <12 weeks with a lower progression in Sharp-van der Heijde score was observed in anti-CCP negative RA (test for interaction p=0.002 without and p<0.001 with adjustments for age, gender, and treatment period). In anti-CCP positive RA a similar, though not significant, tendency was seen with an observed lower rate of destruction in the <12 weeks delay group (test for interaction p=0.07 without, and p=0.18 with adjustments for age, gender, and treatment period).

Similar results were seen for the achievement of sustained DMARD-free remission as were observed for the progression in Sharp-van der Heijde scores. Sustained DMARD-free remission was achieved most frequently in patients with a total delay of <12 weeks (Figure 2). In the <12 weeks delay group, 18.5% (31/168) of patients achieved remission, and in the >12 weeks delay group, 10.5% (41/389) achievement of remission was observed. The hazard ratio for not achieving sustained DMARD-free remission was 1.87 (95%CI 1.18-2.99, p=0.008) for a total delay of ≥12 weeks compared to <12 weeks. The difference did not change after adjusting for age, gender, and treatment period (HR 1.87 (95%CI 1.17-3.00, p=0.009)). Similar results comparing patients with a total delay of <12 weeks and ≥12 weeks were obtained when the analysis was repeated

with the date of the first symptoms as a starting point, both without (HR 1.90, 95% CI 1.19-3.03) and with (HR 1.90, 95%CI 1.18-3.05) correction for age, gender, and year of inclusion. Since in the anti-CCP2 positive subset only 8 patients achieved DMARD-free remission, no stratified analysis was performed.

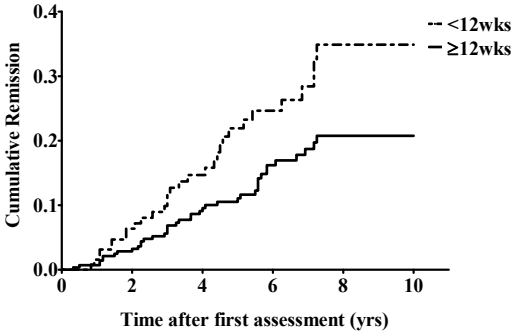

Figure 2. The probability of achieving sustained DMARD-free remission for the different delay categories. Remission as outcome measure for the amount of total delay. Remission was defined as the persistent absence of synovitis for at least one year after cessation of DMARD therapy and the identification of remission by the patient's rheumatologist.[12] Time after first assessment (yrs): follow-up time in years after the first visit to a rheumatologist

Characteristics associated with delay in assessment

Subsequently, patients characteristics associating with an increased delay in assessment were investigated in early arthritis patients (n=1674). Univariate analysis showed that female gender, gradual symptom onset, older age at inclusion, symmetric distribution of symptoms, involvement of small joints and joints of the upper extremities, presence of RF and anti-CCP antibodies, and lower levels of CRP were all significantly associated with a longer duration of total delay ($p<0.001$) (Table 2).

Multivariate regression analysis identified the following variables as independently associated with a longer duration of total delay: older age, gradual symptom onset, involvement of small joints, presence of anti-CCP2 and RF, and lower CRP-levels. As regression analysis was performed on log transformed delay data, the relative estimated progressions were back transformed to the original scale (Table 3). Patient characteristics associated with patient delay and GP-delay showed comparable findings (Table 2 and Table 3).

The findings that the presence of autoantibodies (anti-CCP2 and RF), symmetric involvement of small joints and a gradual onset of symptoms were associated with a longer delay, leads to the presumption that the delay in assessment differs for early arthritis patients with different diagnoses. To study this, early arthritis patients were grouped according to the diagnoses that were achieved within the first year of follow-up and the total delay durations were compared. This showed that reactive arthritis, sarcoidosis and crystal arthritis have the shortest delay in assessment (Figure 3). In contrast, RA patients and patients with psoriatic arthritis and spondylarthropathy had the longest delay in assessment.

Table 2. Baseline characteristics of early arthritis patients associated with patient, GP and total delay in a univariate analysis

		Early arthritis patients					
		Total delay(n=1674)		*GP-delay(n=1111)*		*Patient delay(n=1078)*	
		Weeks (IQR)	p	Weeks (IQR)	p	Weeks (IQR)	p
Gender	*Male*	11.9 (4.4-26.3)	<.001*	6.9 (2.0-16.9)	.001*	2.1 (0.6-6.4)	.049*
	Female	15.3 (6.4-30.7)		8.9 (3.3-19.4)		2.9 (0.8-8.4)	
Age at Inclusion (yrs)	*<52.5*[§]	12.6 (4.0-28.7)	<.001*	6.9 (2.0-18.4)	.001*	2.4 (0.7-8.4)	.907
	≥52.5[§]	15.0 (7.9-28.1)		8.9 (3.9-18.4)		2.6 (0.9-6.6)	
Family history of RA	*No*	13.6 (5.5-28.2)	.119	7.6 (2.6-17.7)	.099	2.4 (0.7-6.9)	.185
	Yes	14.9 (6.0-30.6)		9.3 (3.6-20.9)		2.9 (0.9-8.8)	
Onset of symptoms[‡]	*Acute*	5.6 (1.9-15.9)	<.001*	3.4 (1.0-13.0)	<.001*	0.9 (0.1-2.9)	<.001*
	Subacute	11.8 (5.9-22.0)		7.7 (3.0-14.8)		2.1 (0.9-5.3)	
	Gradual	26.0 (13.6-47.4)		13.0 (6.3-29.4)		5.9 (2.6-16.7)	
Affected Joints	*Small*	16.9 (8.7-32.3)	<.001*	9.1 (3.9-20.6)	<.001*	3.9 (1.0-8.9)	<.001*
	Large	9.7 (2.9-23.4)		4.4 (1.2-15.6)		1.4 (0.3-4.6)	
	Both	13.1 (6.1-26.9)		8.4 (3.0-18.4)		2.0 (0.7-4.6)	
Affected extremities	*Upper*	15.1 (7.6-30.1)	<.001*	8.8 (3.3-19.0)	<.001*	3.1 (1.0-9.1)	<.001*
	Lower	8.6 (2.9-24.6)		4.4 (1.0-15.4)		1.3 (0.3-4.4)	
	Both	14.6 (7.1-27.8)		8.4 (3.0-17.3)		3.0 (0.7-7.9)	
Symmetric distribution affected joints	*Yes*	14.6 (6.9-28.4)	<.001*	9.1 (3.4-19.1)	<.001*	3.0 (1.0-8.4)	.005*
	No	12.6 (4.2-27.8)		6.3 (1.6-16.1)		2.0 (0.4-6.9)	
SJC	*≤5.0*[§]	15.4 (7.9-31.4)	.725	9.1 (3.9-19.2)	.053	3.0 (0.9-8.4)	.286
	>5.0[§]	17.1 (9.7-31.1)		11.1 (4.9-22.5)		2.7 (1.0-7.1)	
Ritchie Score	*<6.0*[§]	15.4 (8.5-30.8)	.674	10.0 (4.4-20.4)	.894	2.9 (0.9-6.8)	.351
	≥6.0[§]	16.9 (8.4-31.3)		10.5 (4.3-21.5)		3.6 (1.0-8.5)	
Anti-CCP2	*Positive*	20.3 (11.6-36.9)	<.001*	12.4 (6.1-22.7)	<.001*	4.3 (1.0-10.9)	<.001*
	Negative	12.7 (4.6-27.1)		6.7 (2.3-16.4)		2.3 (0.7-6.6)	
IgM-RF	*Positive*	18.6 (10.1-35.7)	<.001*	12.3 (5.6-22.7)	<.001*	3.9 (0.9-9.3)	.005*
	Negative	12.3 (4.4-26.6)		6.3 (2.1-16.5)		2.3 (0.7-6.5)	
C-reactive Protein (mg/l)	*<13.0*[§]	16.7 (7.4-32.7)	<.001*	10.0 (3.6-21.9)	<.001*	3.9 (1.0-9.3)	<.001*
	≥13.0[§]	12.1 (4.5-24.1)		7.1 (2.1-15.1)		2.0 (0.6-4.7)	

Delay durations are presented in weeks, median (IQR). The shown p-values reflect the difference within each delay group (total, GP- or patient delay), thus the comparison made is for instance whether the total delay is different between males and females. [§]The continuous variables age, CRP, SJC and Ritchie score were analyzed by creating two groups based on median values. [‡]Defined durations of symptom onset: acute <24 hours; subacute <1 week and gradual ≥1 week. IgM-RF: Rheumatoid factor; CRP: C-reactive protein; SJC: 66-swollen joint count; Ritchie score: 68-tender joint count. *P-value <0.05; Mann-Whitney U/Kruskal-Wallis tests

Table 3. Baseline characteristics of early arthritis patients associated with patient, GP and total delay in a multivariate analysis

Total delay				
		95%CI		
Variable	ratio	lower	upper	p-value
Age at inclusion (yrs)‡	1.004	1.002	1.007	<.001
Female gender§	1.12	1.02	1.22	.014
Gradual onset§	2.22	2.02	2.44	<.001
Involvement of small joints vs. large§	1.31	1.18	1.46	<.001
Involvement of both small and large joints vs. large§	1.16	1.02	1.32	.021
Anti-CCP2§	1.31	1.13	1.51	<.001
IgM-RF§	1.20	1.04	1.37	.010
CRP-level‡	0.995	0.993	0.995	<.001
GP-delay				
		95%CI		
Variable	ratio	lower	upper	p-value
Age at inclusion (yrs) ‡	1.004	1.002	1.009	.004
Female gender§	1.14	1.01	1.29	.040
Gradual onset§	1.93	1.69	2.20	<.001
Symmetric distribution of complaints§	0.79	0.69	0.90	<.001
Anti-CCP2§	1.33	1.09	1.63	.006
IgM-RF§	1.22	1.01	1.47	.039
CRP-level‡	0.995	0.993	0.995	<.001
Patient delay				
		95%CI		
Variable	ratio	lower	upper	p-value
Gradual onset§	2.38	2.09	2.70	<.001
Involvement of joints of lower extremities vs. upper§	0.73	0.63	0.84	<.001
Involvement of joints of both extremities vs. upper§	0.90	0.77	1.04	.155
Anti-CCP2§	1.21	1.04	1.39	.010
CRP-level‡	0.995	0.995	0.998	<.001

The linear regression analysis was performed on log-transformed delay data and the regression coefficients were back transformed for comprehensible results. The inverse log-transformed coefficients represent the estimated relative progression in delay. §In a categorical variable for instance, a ratio of 1.31 (Involvement of small joints vs. large) represents a 1.31 times longer delay. ‡In a continuous variable, a ratio of 1.004 (age at inclusion) indicates a 1.004 times longer delay when there is an increase in age of one year. 95CI: 95% confidence interval; lower: lower bound; upper: upper bound; IgM-RF: Rheumatoid factor; CRP: C-reactive protein

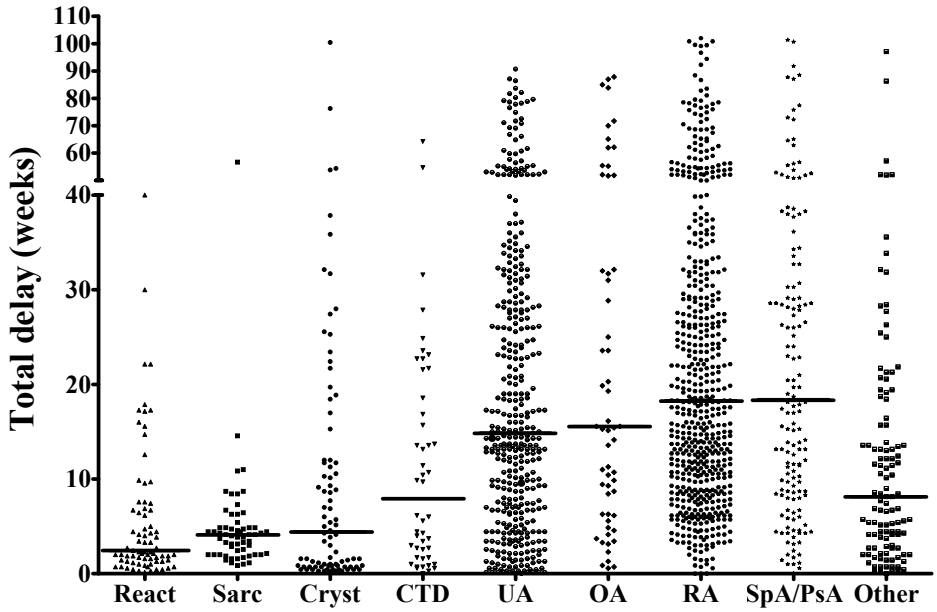

Figure 3. Total delay in assessment by rheumatologists for separate diagnoses. Total delay before visiting a rheumatologist divided per disease category. Depicted are the distribution and median of the total delays per diagnosis (at one year). React: reactive arthritis; Sarc: sarcoidosis; Cryst: crystal arthritis; CTD: connective tissue disease (including SLE and scleroderma); UA: undifferentiated arthritis; OA: Inflammatory osteoarthritis; RA: Rheumatoid arthritis; SpA/PsA: spondylarthropathy/psoriatic arthritis; Other. Horizontal bars represent median delays

DISCUSSION

Early initiation of treatment dramatically improves clinical outcomes in patients with RA. In the last decade, rheumatologists have developed growing awareness of the need to treat early, and this, together with the availability of newer therapies and improved predictive algorithms for patients with early arthritis,[16,17] has improved the outcome of arthritis patients considerably.[18] The present study shows that RA patients who have a delay longer than 12 weeks between first symptoms and visiting a rheumatologist have a worse disease outcome, measured by two outcomes, the rate of joint destruction, and achievement of sustained DMARD-free remission. The effect of delay did not disappear when a more potent treatment strategy was applied after assessment by the rheumatologist. Importantly, amongst all early arthritis patients, patients diagnosed with RA had the longest delay in assessment and the majority of RA patients were assessed after 12 weeks of symptoms, a period which has been referred to as the window of opportunity. These results suggest that, to further improve the outcomes of RA patients, an important challenge is to get patients with arthritis to see a rheumatologist as early as possible after symptom onset.

Diminishing the delay in assessment requires awareness on the part of both patients and their GPs. For that reason, the present study also evaluated which factors associate with the duration of the delay in assessment by a rheumatologist. This revealed that one of the important factors for early presentation to both the GP and to hospital was the acuteness of the start of the complaints. Patients with a gradual symptom onset had a longer delay than patients with an acute or subacute onset of symptoms. Other patient characteristics associated with a longer delay were female gender and an older age. A gender specific delay in referral has been reported before.[19,20] Thus to prevent a worse outcome of arthritis, our findings suggest that attention needs to be focused on the education of patients, in particular the older and female patients, about the significance of their symptoms and the education of GPs to rapidly refer patients, in particular older, female patients with a gradual onset of symptoms.

Several of the patient characteristics that were associated with the duration of delay in assessment of early arthritis patients belong to clusters of variables that are characteristic for specific diagnoses. For instance, an acute onset of symptoms and involvement of large joints of the lower extremities frequently occur in reactive arthritis or sarcoidosis; patients in these diagnostic groups had a short delay. In contrast, a gradual symptom onset and symmetrical involvement of small joints is more common in patients with RA. Both these characteristics and this diagnosis were associated with a longer delay in presentation and referral. Altogether, patients with chronic destructive diseases such as RA, but also psoriatic arthritis and spondylarthropathy, who should be seen particularly early by rheumatologists, had the longest delays in assessment. Therefore the present results underline the importance of putting in place strategies to tackle reasons underlying delay that have been identified at the level of the patient and the GP.[21,22]

Although our findings provide insight into delay in assessment and its association with patient characteristics and disease outcome, the present study has several limitations. Patients were included in the EAC only if they had a symptom duration of <2 years; patients who at first presentation had symptoms for more than 2 years were not studied. However, patients with such a long delay are observed to be very infrequent in our outpatient clinic. Secondly, data were obtained from a single country. In the present study the largest contribution to the total delay was delay in referral by the GP. This is in line with a study from the US[23] and in contrast to recent findings in British cohorts, where the largest contribution to total delay was delay on the part of the patient.[24,25] Differences in health care systems, but also cultural differences[26] could, at least partially, provide an explanation for the contrasting observations. Nevertheless, the median total delay for RA patients was well over 12 weeks in the UK, Canada and in the Netherlands (23 weeks,[24] ~17 weeks[27] and 18.4 weeks respectively) and the present study highlights the consequences of that delay.

The findings that RA patients with a longer delay had more severe joint destruction and less sustained DMARD-free remission are in line with findings that an early initiation of treatment is beneficial to the disease outcome.[8,9] It was questioned whether the patients with a shorter delay had a truly better disease course or were just seen earlier in the disease course, resulting in

a seemingly lower level of joint destruction. Therefore, analyses were repeated with the date of the first complaints as a starting point. This showed that patients who had a delay of <12 weeks indeed developed less severe disease compared to patients with a longer delay.

There are two potential explanations for the observed difference in severity between the <12 weeks and ≥12 weeks delay groups. First, it may be that RA patients that were assessed in a short time constitute a subset of RA that by itself is characterized by a better outcome. It is known that the subset of RA characterized by the absence of anti-CCP antibodies has a better disease outcome than the anti-CCP positive subset,[14] and in our data anti-CCP positive patients had more often a gradual onset of complaints (49.3% vs. 38.2%) and more delay (22.0 vs. 14.3 weeks, median) than anti-CCP negative RA patients. To account for such differences between RA patients, the effect of delay was studied in both the anti-CCP positive and anti-CCP negative subset. This showed a significant association between delay in assessment and joint destruction in anti-CCP2 negative RA patients and a similar tendency in anti-CCP positive RA patients. The present data however do have insufficient power for these sub-analyses and more specifically do not allow making definite conclusions on the effect of delayed assessment on joint destruction in the subset of anti-CCP positive patients. Alternatively, patients assessed within 12 weeks were treated earlier which may have contributed to a less severe course of RA which is in line with previous data,[4] and supports the hypothesized existence of a window of opportunity. Nonetheless, regardless of the explanation of the findings (better outcome in anti-CCP negative patients with a more acute symptom onset or better outcome due to early initiation of treatment) the main argument to refer as early as possible is that it provides the opportunity to modify RA in an early phase with potential beneficial effects on the future disease course.

In conclusion, a shorter time to assessment by a rheumatologist is associated with more DMARD-free remission and less joint destruction in RA. Despite this association, among all early arthritis patients, those diagnosed with RA had one of the longest delays in assessment and only one third was assessed within the so-called window of opportunity. Since rheumatologists are nowadays aware on the importance to treat early, our results suggest that in order to further improve disease outcomes in RA it will be crucial to diminish the delay in assessment by a rheumatologist. Further work could test whether accelerated treatment strategies indeed leads to improve disease outcomes in RA.

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CHAPTER 10

The window of opportunity in ACPA-positive rheumatoid arthritis is not explained by ACPA characteristics

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Very early therapy of rheumatoid arthritis (RA) with disease-modifying anti-rheumatic drugs is associated with lower levels of joint destruction and a higher chance on achieving remission.[1-5] Having symptoms for >12 weeks at treatment initiation is a strong and independent risk factor for a persistent disease course.[1,3-5] These observations have led to the concept of the 'window of opportunity'.[2] This hypothesis presumes that underlying disease processes are not fully matured in the very early stage of arthritis, making modulation more successful. However, putative biological mechanisms remain unexplored.

Anti-cyclic citrullinated protein antibodies (ACPA) precede arthritis development and are associated with a severe disease course.[6] We hypothesized that the ACPA-response broadens within the very early phase of RA and in doing so limits the 'window of opportunity'. Therefore it was examined whether patients that are assessed within 12 weeks of symptom onset have a less broadened ACPA-response than patients with longer symptom duration.

309 ACPA-positive patients (defined by anti-CCP2-positivity) fulfilling the 1987-ACR criteria for RA and included in the Leiden Early Arthritis Clinic[7] were studied on the association between symptom duration and the progression in joint destruction over 7.5 years, with symptom onset as starting point.[3] Yearly radiographs of hands and feet were scored according to the Sharp-van der Heijde method.[7] A repeated measurement analysis was used with a random person and time effect; the fixed effect of time was modeled with linear spline functions with knots at each year.[3] Adjustments were made for age, gender and treatment strategy. RA patients that presented with <12 weeks or ≥12 weeks of symptoms were compared for level, isotype-usage and fine specificity of ACPA at inclusion. Antibody reactivity against peptides derived from human proteins (the citrullinated (Cit) and the uncitrullinated form of two linear peptides derived from vimentin (Vim1-16:STCitS VSSS SYCitCit MFGG and Vim59-74:VYAT CitSSA VCitLCit SSVP), two linear peptides derived from fibrinogen (Fibα27-43:FLAE GGGV Cit GPR VVER H and Fibβ36-52:NEEG FFSA CitGHR PLDK K), one linear peptide derived from α-enolase (Eno5-20:KIHA CitEIF DSCitG NPTV) and Myelin Basic Protein (MBP)) were determined by ELISA and described previously.[3,7-9] Anti-CCP3 and anti-MCV were also measured by ELISA (Quanta Lite CCP version 3.1 for IgG/IgA, Inova Diagnostics San Diego, USA and Orgentec Diagnostika, Mainz, Germany).

RA patients that presented <12 weeks of symptom onset had less progression in joint destruction over 7.5 years (p=0.04) (Figure 1). Patients with symptoms <12 weeks revealed no differences in anti-CCP2 level, isotype usage or fine-specificity recognition profile compared to patients with longer symptom duration (Table 1).

To our knowledge this is the first study investigating ACPA-characteristics in relation to the so-called 'window of opportunity'. Recently published data showed a trend for less joint destruction in ACPA-positive RA patients presenting with symptoms <12weeks.[3] In the present study the radiographic data were extended. No clear differences were observed with respect to ACPA-characteristics in relation to symptom duration. Although it cannot be excluded that other ACPA-characteristics, such as glycosylation patterns or other 'fine-specificities', would show

Table 1. ACPA characteristics at inclusion of ACPA-positive RA patients with symptoms for <12 or ≥12 weeks

	<12 weeks		≥12 weeks			
Anti-CCP2 levels*					P-value	
Median (AU) IQR	766 285-1711		642 215-1560		0.5	
Fine-specificity	<12 weeks		≥12 weeks		OR	95% CI
cVim1-16- cVim1-16+	54 8	87.1% 12.9%	177 26	87.2% 12.8%	0.99	0.42-2.32
cVim59-74- cVim59-74+	30 32	48.4% 51.6%	100 106	48.5% 51.5%	0.99	0.56-1.75
cFib-α – cFib-α +	40 22	64.5% 35.5%	156 50	75.5% 24.3%	0.58	0.32-1.07
cFib-β – cFib-β +	13 48	21.3% 78.7%	60 136	30.6% 69.4%	0.61	0.31-1.22
cEno5-20 – cEno5-20 +	40 22	64.5% 35.5%	139 67	67.5% 32.5%	0.88	0.48-1.59
MBP – MBP +	19 43	30.6% 69.4%	74 132	35.9% 64.1%	0.79	0.43-1.45
MCV – MCV +	3 58	4.9% 95.1%	10 191	5.0% 95.0%	0.99	0.26-3.71
CCP3 – CCP3 +	2 59	3.3% 96.7%	14 188	6.9% 93.1%	0.46	0.10-2.06
0-4 peptides** 5-8 peptides**	24 35	40.7% 59.3%	97 92	51.3% 48.7%	0.65	0.36-1.18
ACPA isotype usage***					OR	95% CI
IgM-ACPA – IgM-ACPA +	13 26	33.3% 66.7%	52 102	33.8% 66.2%	0.98	0.47-2.07
IgA-ACPA – IgA-ACPA +	14 24	35.9% 64.1%	50 104	32.5% 67.5%	1.17	0.56-2.43
IgG1-ACPA – IgG1-ACPA +	0 39	0% 100%	2 152	1.3% 98.7%	N/A	N/A
IgG2-ACPA – IgG2-ACPA +	3 36	7.7% 92.3%	26 128	16.9% 78.0%	0.41	0.12-1.43
IgG3-ACPA – IgG3-ACPA+	16 23	41.0% 59.0%	63 91	40.9% 59.1%	1.01	0.49-2.05
IgG4-ACPA – IgG4-ACPA +	0 39	0% 100%	6 148	3.9% 96.1%	N/A	N/A
0-4 isotypes 5-6 isotypes	14 25	35.9% 64.1%	55 99	35.7% 64.3%	1.01	0.49-2.10

Fine-specificity data were assessed in patients included between 1993 and 2006. Fine-specificity data were missing for 61 patients. Isotype data were determined previously in patients included between 1993 and March 2004 and are therefore missing in 116 patients. *Difference in anti-CCP2 levels was analyzed using Mann-Whitney test. IQR=interquartile range. cVim=citrullinated vimentin; cFib=citrullinated fibrinogen; cEno5-20=citrullinated Enolase 5-20; MBP=myelin basic protein; MCV=mutated citrullinated vimentin.**8 peptides were included for the high versus low recognition analyses: cVim1-16, cVim59-74, cFib-α, cFib-β, cEno5-20, MBP, MCV, CCP3. ***ACPA isotypes were measured using anti-CCP2 peptides

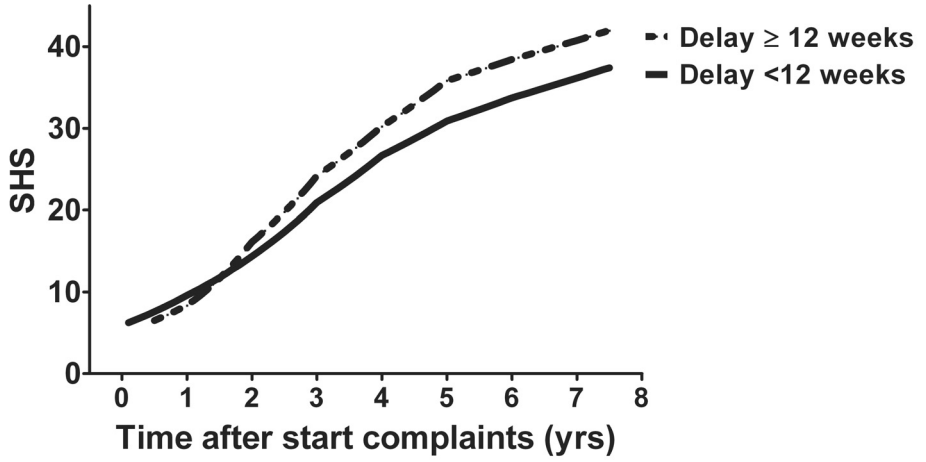

Figure 1. Joint destruction (Sharp/van der Heijde scores) over time in ACPA-positive RA patients with <12 or ≥12 weeks of symptoms at first presentation at the rheumatologist. The date of symptom onset is used as starting point. 70 ACPA-positive patients (22.7%) presented <12 weeks (median after 8 weeks of symptoms) and 239 ACPA-positive patients presented after ≥12 weeks of symptoms (median symptom duration at first presentation at 27 weeks). The RA patients studied were included in the Leiden Early Arthritis Clinic between 1993 and 2006

differences, our data indicate that the 'window of opportunity' is not reflected in the maturation of the ACPA-response.

A longitudinal study-design with regular assessments of ACPA-characteristics within the same patients would be more appropriate than a cross-sectional study. However, as ACPA-positive RA patients often present relatively late (only 22.7% of the ACPA-positive RA patients visited a rheumatologist <12 weeks of symptom onset), it will be difficult to obtain adequate patient numbers.

In conclusion, ACPA-positive RA patients with symptoms <12 weeks have less progressive disease than patients with a longer symptom duration. However, the broadness of the ACPA-response is not different between these groups; indicating that maturation of the autoantibody response occurs even earlier.[10]

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PART V

Subphenotypes of RA severity

CHAPTER 11

Repair of joint erosions in rheumatoid arthritis: prevalence and patient characteristics in a large inception cohort

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ABSTRACT

Background

Joint destruction in rheumatoid arthritis (RA) was until recently seen as an irreversible state. Lately, it was defined that repair of bone erosions occurs; however little is known about its prevalence. This study investigates the frequency of repair and patients characteristics associated with repair in an inception cohort.

Patients and methods

250 RA patients, included in the Leiden Early Arthritis Clinic between 1993-2000 and treated with conventional DMARD-therapy, were studied (mean follow-up 10.1 years). Yearly made radiographs were scored using the Sharp-van der Heijde method, initially aware of the chronology. Patients with a negative change in erosion scores on subsequent radiographs were selected and their series of radiographs were rescored with concealed time sequence by three readers. Repair was defined as agreement of two readers in having a negative change in erosion scores that persisted for at least two years.

Results

Repair was identified in 32 joints in 18 patients (7.2%). Patients with repair had more frequent autoantibodies (RF, ACPA) and a higher level of joint destruction. In the joints with repair arthritis was absent in the two years preceding repair.

Conclusions

Repair occurred in 7.2% of the RA patients, particularly in clinically inactive joints in patients with severe destructive disease.

INTRODUCTION

Rheumatoid arthritis (RA) often results in destruction of bone and cartilage, visualized on radiographs as erosions and joint space narrowing respectively. For a long time the bone damage was considered to be permanent.[1] Recently some studies sustained the possibility of radiological repair.[2-6] Dedicated research in the context of Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT),[7,8] along with recent literature reviews,[9,10] led to the conclusion that "repair does exist". This is of utmost clinical relevance because it demonstrated that the "repair machinery" is able to take away, at least partly, the consequences of damage by RA. If the biological basis of this phenomenon could be understood, it would allow the development of therapies specifically targeted to stimulate these repair mechanisms. This study aims to assess the frequency of repair in a large inception cohort of RA patients treated with conventional disease modifying antirheumatic drugs, and to characterize the patients expressing repair.

PATIENTS AND METHODS

Patients

250 RA patients, consecutively included in the Leiden Early Arthritis Clinic (EAC) between 1993 and 2000 were studied. The era 1993-2000 was chosen as it has the longest duration of follow-up (mean 10.1 years, SD=2.3), resulting in a real opportunity to express repair. Clinical and laboratory characteristics were yearly measured and radiographs of hands and feet were yearly taken.[11] Treatment strategies differed per inclusion period. Patients included between 1993-1995 were treated with delayed therapy (initially analgesics, subsequently chloroquine or salazopyrin) and between 1996-2000 with prompt initiation of chloroquine, salazopyrin or methotrexate. Biologicals or aggressive combination therapy were not applied.

Radiograph scoring

The radiographs were scored using the Sharp–van der Heijde method[12] by one reader, blinded to the clinical data, initially aware of the chronology. Patients with a negative change in erosion scores on subsequent radiographs were selected. Their series of radiographs were mixed with series of patients with stable or positive change in erosion scores, so that the readers were unaware of the scores that were assigned previously. These radiographs were rescored with concealed time sequence by three trained readers. The intrareader intraclass correlation coefficient for the status scores was 0.91. The intraclass correlation coefficient between reader 1 and 2 was 0.94, between reader 1 and 3 0.95 and between reader 2 and 3 0.93.

Definition of repair

Repair was defined as fulfilling all of the following three criteria a) presence of a negative change in erosion score on a joint level on two subsequent time points both when scored with known

and concealed time order, b) persistency of the lower erosion score ≥2 subsequent years, c) agreement on the negative change in erosion score between at least two of the three readers. In case data on two subsequent years were not available, all three readers had to agree with the negative change in erosion score.

Patient characteristics

Patients with and without repair were compared for several baseline characteristics and for the total Sharp-van der Heijde scores during follow-up. The achievement of sustained DMARD-free remission (the persistent (≥1 year) absence of arthritis after cessation of therapy with disease modifying antirheumatic drugs)[13] was evaluated in both groups. The annually assessed swollen joint count was studied in order to investigate whether the joints that showed repair had clinically detectable arthritis in the two years preceding the development of radiologically visible repair.

Statistical analysis

Differences in means were analyzed with the Mann-Whitney test. Proportions were compared using the chi-square test. The Statistical program for Social Sciences (SPSS) version 14 was used. P-values <0.05 were considered significant.

RESULTS

Prevalence of repair

Seventy of 250 RA patients had at least once a decrease in erosion score in any of the joints, evaluating all series of radiographs with known time-order. After rescoring with concealed time-order, 32 joints with repair were identified in 18 (7.2%) patients. Of these, 26 concerned small joints of the hands (8 MCP joints, 9 PIP joints and 9 radiocarpal joints) and 6 concerned MTP joints. Thirty joints showed persistency of the negative change in erosion score for ≥2 years and for 2 joints no data on two additional years were available but there was agreement of all three readers in the identification of repair. 61% of the patients showed repair in one joint; 11%, 17% and 11% expressed repair in 2, 3 and 4 joints respectively. The highest frequency of repair occurred after 4 to 6 years follow-up (Figure 1). The frequency of repair was 13.0% for inclusion between 1993 and 1995 and 5.2% for inclusion between 1996 and 2000.

Baseline characteristics of patients expressing repair

Patients with and without repair revealed no difference in age, gender, Ritchie score, swollen joint count, CRP level and total Sharp-van der Heijde score at baseline (Table 1). In contrast, patients with repair were more often RF-IgM positive (OR 3.7, 95%CI 1.2-11.5, p=0.025) and anti-CCP positive (OR 7.9, 95%CI 1.8-35.2, p=0.007) compared to the non-repair group.

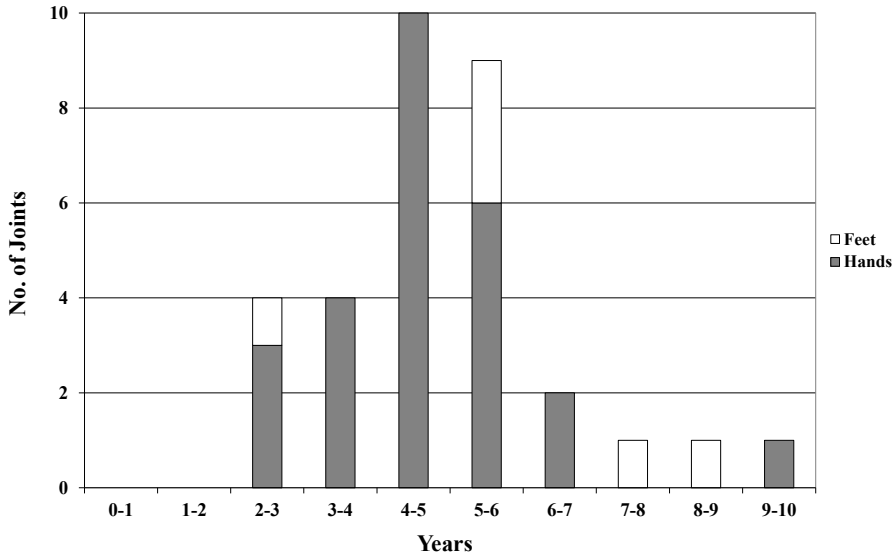

Figure 1. Frequency of repair during the years of follow-up, indicated for small joints of the hands and the feet separately. The total number of small joints assessed in the hands is 32 and in the feet is 12. The joints assessed in the hands are the proximal inter-phalangeal (PIP) joint in digits 1 to 5, the metacarpo-phalangeal (MCP) joint in digits 1 to 5 and 6 radio-carpal sites (base of metacarpal bone digit 1, trapezium, lunate, scaphoid, distal ulna and distal radius and in the feet are the inter-phalangeal (IP) joint digit 1 and metatarso-phalangeal (MTP) joint in digits 1 to 5

Table 1. Baseline characteristics of patients with and without repair

	Repair group N=18	Non-repair group N=232	P value
Age at baseline, mean (SD)	59.3 (9.3)	55.1 (16.9)	0.30
Female gender, No (%)	13 (72)	155 (67)	0.67
Ritchie score, mean (SD)	11.5 (8.0)	10.8 (7.8)	0.73
44 Swollen Joint Count, mean (SD)	5.9 (2.4)	6.0 (3.4)	0.86
ESR in mm/h, mean (SD)	44.2 (25.0)	41.6 (29.9)	0.68
CRP in mg/l, mean (SD)	26.4 (21.3)	29.4 (28.2)	0.59
RF-IgM positive, No (%)	14 (77.8)	112 (48.7)	0.025
Anti-CCP2 positive, No (%)	15 (88.2)	106 (48.8)	0.007
Total Sharp score, mean (SD)	8.1 (6.1)	7.5 (9.1)	0.79

CCP, cyclic citrullinated peptide; CRP, C-reactive protein; ESR, erythrocyte sedimentation date; RF, rheumatoid factor.

Disease course of patients expressing repair

Seventeen patients with repair (94%) had an increase in total Sharp score at the same time as showing repair in individual joints; only one patient showed a decrease in total Sharp-score, indicating that, next to repair, simultaneous progression was present in other joints.

During the disease course patients with repair had significant higher Sharp-van der Heijde scores compared to patients without repair (Figure 2A). A similar observation was done for the total erosion score (Figure 2B).

The achievement of sustained DMARD-free remission was compared for patients with and without repair. One patient of the repair group had clinical remission (5.5%), compared to 16 % (56 out of 232 patients) in the non-repair group (OR 0.15, 95%CI 0.01-1.37, p=0.07).

The presence of joint swelling for the 23 joints showing repair in the MCP, PIP or MTP-joints was evaluated at the two previous years. This showed that joint swelling was absent in 22 joints in two years preceding repair and in 1 joint swelling was absent one year preceding repair.

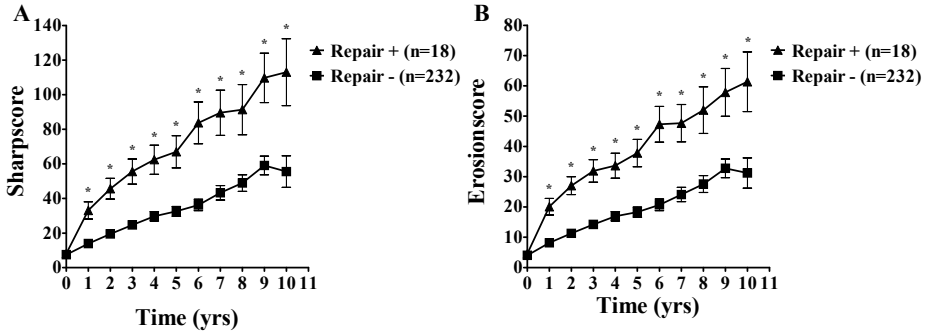

Figure 2. Total Sharp-van der Heijde scores (mean ± SEM) (A) and total erosion scores (mean ± SEM) (B) during follow-up in patients with and without repair. From the patients with repair, the number of radiographs available per year were: 18 at baseline, 18 after 1 year of follow-up, 18 after 2 years of follow-up, 15 after 3 years of follow-up, 14 after 4 years of follow-up, 17 after 5 years of follow-up and 12, 12 , 10, 8 and 8 after 6-10 years of follow-up respectively. *p<0.05

DISCUSSION

The present study investigated repair in an inception cohort with a long duration of follow-up. Previous studies concerned data from clinical trials or evaluated a selected set of RA patients.[2,5,6,14] Importantly, these studies formed the basis for the acceptance of the existence of repair. We now report on the prevalence in a large longitudinal cohort of RA patients treated with conventional treatment strategies. Our results show that, despite the absence of aggressive or biological anti-rheumatic therapy, repair occurs in part of the general RA population.

The prevalence of repair observed here (7.2%) is somewhat lower than reported previously (10.7%).[14] We have chosen a strict definition of repair to reduce the chance on false-positive findings; this may indicate that our prevalence is an underestimation. In addition, the comparison of erosion scores of individual joints between two consecutive time-points may have introduced misclassification, in some cases repair would have been more easily detected in case a larger interval between the radiographs was compared. Third, the finding of a lower prevalence may be caused by the fact that we studied a general RA population and not a selection of RA patients.

Interestingly, repair occurred preferentially in patients with severe joint destruction. This might seem surprising as it could be hypothesized that repair will predominantly be present in the patients with a low total level of joint destruction. Several possibilities may explain this observation. First, it may be a methodological issue based on the presumption that a refill is more easily detected in large erosions. If this is true, repair should predominantly be present in joints with a high erosion score. Our data are not supportive for this notion. The erosion score for individual joints ranges between 0 (no erosion) and 5 (maximum score). The majority of patients with repair showed a decrease in the erosion score from 2 till 1 or from 1 till zero, and thus did not reveal repair in joints that are particularly severely damaged. A second possibility is again methodological. In patients with a lot of damage, many joints show erosions and therefore in these patients more joints are 'at risk' for showing repair. A third possible explanation is biological. In general the human body tends to heal destruction and aims for homeostasis. It can be hypothesized that the more destruction is present, the more regenerating processes are activated. Then after the inflammation or the processes that drive the destruction of bone are disappeared, the enhanced regenerating mechanisms may result in repair.

At the same time repair occurred in some joints, the total Sharp-van der Heijde score increased, indicating progression in other joints. This is in concordance with a study performed by the OMERACT group,[4] and implies that repair is a localized process. The observed absence of joint swelling in the two years preceding repair is in line with similar findings in the TEMPO-trial.[15]

In conclusion, repair occurs in 7.2% of conventionally treated RA patients, particularly in clinically inactive joints in patients with severe destructive disease. Further studies on the biological basis of repair are challenging as they may allow the development of therapies specifically targeted to stimulate these repair mechanisms.

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CHAPTER 12

Joint damage in response to inflammation in rheumatoid arthritis; unraveling underlying mechanisms using extreme discordant phenotypes

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Submitted

ABSTRACT

Introduction

The relation between joint inflammation and destruction is characteristic for RA. Individual patients differ in the amount of joint damage in response to inflammation; the mechanisms underlying coupling/uncoupling are incompletely understood. Evaluation of patients with extreme erosive responses to local inflammation may increase our comprehension. This study explored whether this approach is feasible.

Methods

RA patients included in the Leiden Early Arthritis Clinic with complete 5 years follow-up data (n=159) were studied. Yearly visits included radiographs of hands and feet and swollen joint counts (SJC). The cumulative inflammatory burden was expressed with an AUC of SJCs. Patients with high-inflammatory non-erosive ('resistant') and low-inflammatory high-erosive ('sensitive') phenotypes were identified.

Results

Six patients (4%) had a resistant phenotype; these were rheumatoid factor negative and had short symptom duration. Seventeen patients (11%) had a sensitive phenotype; these patients had a lower SJC at baseline and were often rheumatoid factor positive. Power analyses performed with different risk factor frequencies, different levels of significance and the number of extreme patients identified yielded powers >80%.

Conclusion

Patients with extreme erosive responses to local inflammation were identified. Further evaluations on these patients may elucidate mechanisms contributing to the connection of inflammation and destruction of joints in RA.

INTRODUCTION

Understanding of the mechanisms involved in disease progression or resistance to progression is required to derive strategies to diminish such progression. Generally a whole population of patients is studied to identify such factors. Alternatively, the most and least progressive patients can be compared. This extremes-of-the-phenotype approach reduces the number of patients that need to be studied; this is beneficial when it is impractical or expensive to determine risk factors in large numbers of patients. A third approach, the extreme-discordant-phenotype methodology, studies the response of individuals on an increasing dose of stimuli; the extremes of this gradient are identified as 'sensitive' or 'resistant' phenotypes.[1,2]

This extreme-discordant-phenotype methodology has been successful in the identification of genetic variants involved in responsiveness to drugs, malignancies, and infectious diseases.[3-5] An example of a 'sensitive phenotype' is the observation that some patients with malignancies developed severe toxicity after receiving 5-fluorouracil. Thorough evaluation of these patients led to the association with a complete deficiency of dihydropyrimidine dihydrogenase activity in peripheral blood mononuclear cells, which is caused by diverse genetic alterations.[5] An outstanding example of the identification of a resistant factor is based on the observation that some individuals highly exposed to HIV never developed the infection. This resulted in the identification of a deletion in the gene encoding the chemokine coreceptor CCR-5, which is now a drug target.[4]

Inflammation and destruction of joints are hallmarks of Rheumatoid Arthritis (RA) and the notion that local inflammation leads to destruction of joints is basic to the concept of RA. On the group level, the amount of inflammation is indeed correlated with the amount of erosive joint damage. However, the degree of erosiveness in response to inflammation is highly variable between RA patients and also disconnection has been observed.[6-11] The mechanisms underlying such coupling/uncoupling are incompletely understood. Since the readiness for bone to erode in response to local inflammation appears to be an individual's characteristic, genetic factors may play a role.

Our ultimate aim is to unravel processes contributing to an individual RA-patient's predisposition to develop joint erosions in response to local inflammation. In this study we evaluate whether the extreme-discordant-phenotype methodology is feasible to this end.

PATIENTS AND METHODS

Patients

RA patients included in a population based inception cohort, the Leiden Early Arthritis Clinic (EAC), were studied. For an extensive cohort description see reference.[12] Written informed consent was obtained from all participants. The study was approved by the local medical ethics committee. All RA patients satisfied the 1987 ACR-criteria for RA. From the total number of 695 RA patients, 441 RA patients had achieved 5 years of follow-up. Of these, 159 RA patients

had missed none of the yearly follow-up visits and had complete follow-up data during 5 years. Baseline characteristics were not significantly different between patients with and without missing follow-up visits (data not shown). The 159 RA patients were studied to identify patients with high-inflammatory non-erosive ('resistant') and low-inflammatory high-erosive ('sensitive') phenotypes.

Joint damage

All 1908 hand and feet radiographs were scored by one experienced reader (MPMvdL) according to the Sharp-van der Heijde score (SHS) in chronological order. 499 radiographs were rescored; the interclass-observer correlation coefficient was 0.91. The total erosion SHS was used. Based on previous findings,[13] patients whom had a SHS erosionscore ≤1 after 5 years were defined as having non-erosive disease. To select the patients with a high-erosive disease course, the patients with the highest quartile of SHS erosionscores at the 5-years visit were evaluated.

Joint inflammation

Local inflammation of the joints was assessed by the 44-swollen joint count (SJC) at each visit. For classification as 'high-inflammatory' synovitis had to be observed almost persistently during the follow-up period; a SJC of 0 was allowed at only one point in time. For classification of 'low-inflammatory' the SJC during follow-up required to be 0 in three out of the five follow-up time-points and to be ≤5 at the other follow-up timepoint(s). These cut-off values are arbitrary and were chosen based on visual evaluation of the SJCs of the whole RA-population during the follow-up visits. To appraise whether these cut-offs allowed the identification of extreme discordant phenotypes, for each patient the cumulative inflammatory burden over 5 years was estimated by calculating an area under the curve (AUC) and plotted against the SHS-erosionscore at 5 years.

In the evaluation on joint inflammation above, 44 joints (44 SJC) were studied. Although it was observed that in the present dataset the SJC was mainly driven by the number of inflamed small joints, a comparison of joint destruction in the small joints with joint inflammation in small and large joints may be considered inequitable. Therefore we also evaluated inflammation in 32 small joints (the wrist, MCPs, PIPs and MTPs joints that were assessed in the SHS) and again identified patients that were 'high inflammatory' and 'low inflammatory' using the cut-off values as described above.

Statistical analysis

Patient characteristics were compared using crosstabs and Chi-square, Fisher exact, Mantel-Haenszel statistics in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Power calculations were performed for testing two independent proportions based on the Z-test in Pass 2008 (NCSS, LCC Kaysville, Utah, USA), using two significance levels namely 0.05 and 0.005.

RESULTS

Extreme discordant phenotypes

Applying the definitions of non-erosive, high-erosive, low-inflammatory and high-inflammatory as indicated, resulted in the selection of 6 RA patients with a high-inflammatory non-erosive ('resistant') phenotype and 17 patients with a low inflammatory high-erosive ('sensitive') phenotype (Figure 1A). The remaining 136 patients (85%) were labeled as the reference group. The AUC of the SJC over time was plotted against the erosion score at 5-years (Figure 1B); the patients with the 'sensitive' and 'resistant' phenotype are indicated in red and blue respectively.

Evaluations of inflammation in small and large joints and joint damage in only small joints may be imbalanced when inflammation is predominantly present in large joints. To explore this, analyses were repeated comparing inflammation and destruction in small joints only. Then, 6

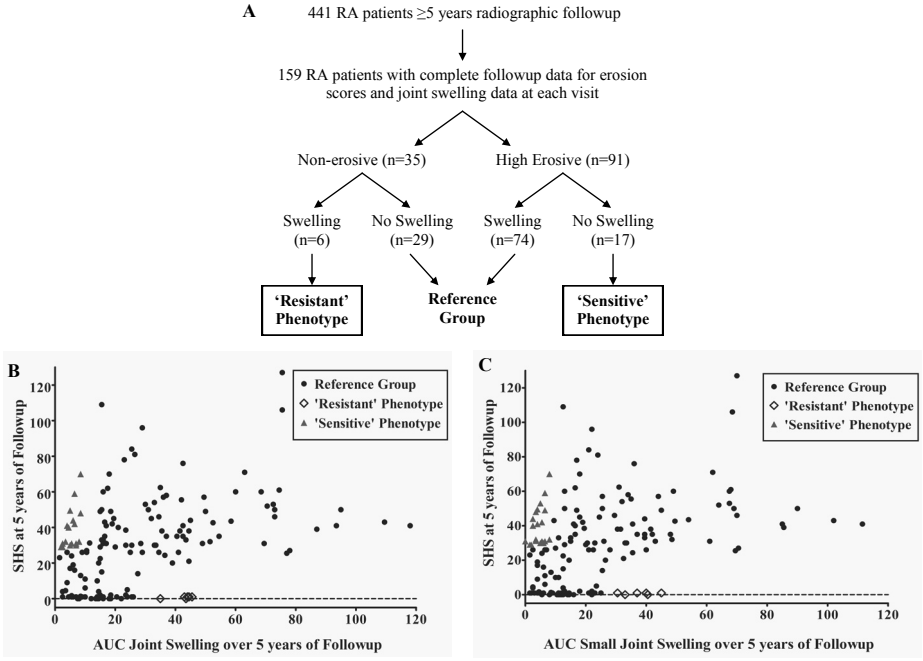

Figure 1. Flow diagram of the selection of extreme discordant phenotypes (A), graphic representation of the cumulative level of inflammation and damage over 5 years of disease evaluating inflammation in 44 joints (B) and evaluating inflammation of small joints only (C). As shown in panel A, patients were used for analysis if they had complete radiographic and SJC data during 5 years of follow-up. Patients were non-erosive when having SHS erosion score ≤1. High-erosive patients were within the highest quartile of erosions scores at year 5. In high-inflammatory patients synovitis had to be present in almost all visits as a SJC of 0 was allowed only at one point in time. Low-inflammatory patients the SJC was 0 for at least three out of five follow-up time points and ≤5 at the other followup time points. For Figure 1A and 1B inflammation was assessed in 44 joints, for Figure 1C inflammation was assessed in 32 small joints (wrists, MCPs, PIPS, and MTP joints that are also evaluated on joint destruction in the Sharp/van der Heijde method)

RA patients were identified with a high-inflammatory non-erosive ('resistant') phenotype and 20 patients with a low-inflammatory high-erosive ('sensitive') phenotype (Figure 1C). The patients with the resistant phenotype were the same individuals as in Figure 1B. Also the patients with the sensitive phenotype were similar but extended with three additional patients. These three additional sensitive patients were "low-inflammatory" when assessing small joints only, but not when assessing 44 joints as they had inflammation in large joints. Therefore, to study the most extreme discordant patients, patients that in both analyses were identified as extreme discordant were evaluated in further analyses.

Patient characteristics

Baseline characteristics of the patients with the 'sensitive' or 'resistant' phenotype were compared to that of patients in the reference group (Table 1). Compared to the reference group, patients with the 'resistant phenotype' were characterized by the absence of IgM-rheumatoid factor, a low frequency of anti-CCP2-positivity, more frequently an acute onset of symptoms, a shorter symptom duration and a higher SJC at first presentation. Patients with the 'sensitive phenotype' were more often rheumatoid-factor positive, had a longer symptom duration and a lower SJC at baseline than the reference group. The erythrocyte sedimentation rate and C-reactive protein (CRP) levels at baseline were not statistically different between the 'sensitive', 'resistant' or reference groups.

Power of extreme discordant phenotype approach

Next we aimed to evaluate the power to identify genetic variants associating with these phenotypes, using the number of patients identified. Our hypothesis is that these extreme discordant phenotypes are multi-factorial and caused by either recessive effects of common genetic variants or rare genetic variants. For the recessive effect we assume that the penetrance is not 100%, i.e. that also in the reference group recessive genotypes occur. Concerning rare variants it is assumed that multiple deleterious and neutral variants are present in the studied genomic region.[14] For comparisons of the number of patients carrying the recessive genotype or the number of patients carrying rare mutations between the two groups the same Z-test can be used; hence one power study is required applying to both situations. For the non-erosive group, the power to detect differences between the high-inflammatory (n=6, 'resistant') and non-high-inflammatory group (n=29) was determined. For an α of 0.005 and for instance carrier frequencies of 0.83 (5 out of 6) in group 1 (P1) and 0.03 (1 out of 26) to 0.10 (3 out of 26) in group 2 (P2), the power to detect a difference is above 90% (Figure 2A). When using an α of 0.05 and similar frequencies, the power is 97% (Figure 2B). For the high-erosive group, the power to detect differences between the low-inflammatory (n=17, 'sensitive') and non-low-inflammatory group (n=74) was determined. In case of carrier frequencies of 0.8 and 0.1, the power to detect a difference is 100%, both for α's of 0.005 and 0.05 (Figure 2C, D). The power for other genotype frequencies is depicted in Figure 2. Overall, it was observed that evaluations on the present number of patients with extreme discordant phenotypes and rare genetic variants have sufficient power.

Table 1. Characteristics at baseline of all patients, and the patients in the 'sensitive', 'resistant' and reference group

	All patients n=159	Reference group (n=136)	'Sensitive' Phenotype (n=17)	'Resistant' phenotype (n=6)
Female, n (%)	106 (66.7)	90 (66.2)	10 (58.8)	6 (100)
Age at inclusion (yrs), mean (SD)	55.2 (13.8)	55.1 (13.8)	57.5 (13.9)	51.3 (13.8)
Symptom duration at first presentation, weeks mean (SD)	31.9 (26.8)	32.1 (26.5)	37.9 (31.6)	16.1 (20.2)
< 6 weeks, n(%)	36 (24.8)	32 (25.2)	1 (5.9)	3 (50.0)
≥ 6 weeks, n (%)	109 (75.2)	95 (74.8)	11 (91.7)	3 (50.0)
Onset of symptoms				
(Sub)Acute	76 (50.7)	64 (48.5)	8 (61.5)	4 (80.0)
Gradual	74 (49.3)	68 (51.5)	5 (38.5)	1 (20.0)
Morning stiffnes (min), mean (SD)	89.6 (97.7)	88.5 (86.2)	92.5 (171.6)	105.0 (92.5)
44 Swollen joint count, mean (SD)	10.1 (7.7)	10.5 (7.7)	5.2 (3.3)	14.8 (10.1)
1 medium-large joint, n (%)	3 (1.9)	1 (0.7)	1 (5.9)	1 (16.7)
2-10 medium-large joints, n (%)	3 (1.9)	3 (2.2)	0 (0)	0 (0)
1-3 small joints, n (%)	19 (11.9)	15 (11.0)	4 (23.5)	0 (0)
4-10 small joints, n (%)	54 (34.0)	44 (32.4)	9 (52.9)	1 (16.7)
> 10 joints, n (%)	80 (50.3)	73 (53.7)	3 (17.6)	4 (66.7)
ESR (mm/hr), mean (SD)	46.1 (32.7)	46.1 (32.7)	46.3 (32.4)	46.3 (38.1)
CRP (mg/l), mean (SD)	36.6 (40.9)	34.7 (40.4)	52.6 (44.1)	40.7 (43.2)
IgM-RF-positive, n (%)	101 (64.7)	88 (64.7)	13 (92.9)	0 (0)
Anti-CCP2-positive, n (%)	103 (66.5)	91 (67.9)	11 (73.3)	1 (16.7)
HAQ, mean (SD)	0.91 (0.68)	0.92 (0.66)	0.66 (0.88)	1.14 (0.70)

Comparison of 'resistant' phenotype versus reference group: IgM RF p=0.003, anti-CCP p=0.018, symptom duration p=0.057, onset of symptoms p=0.2 and SJC p=0.2. Comparison of 'sensitive phenotype' versus reference group: IgM-RF p=0.036, SJC p=0.004

DISCUSSION

The relation between inflammation and subsequent joint damage is characteristic for RA and is basic to current treatment strategies that aim to prevent or retard joint damage by reducing the inflammatory load. Although this strategy is effective on the group-level, the coupling between inflammation and destruction of joints in individual patients is variable. One method to identify

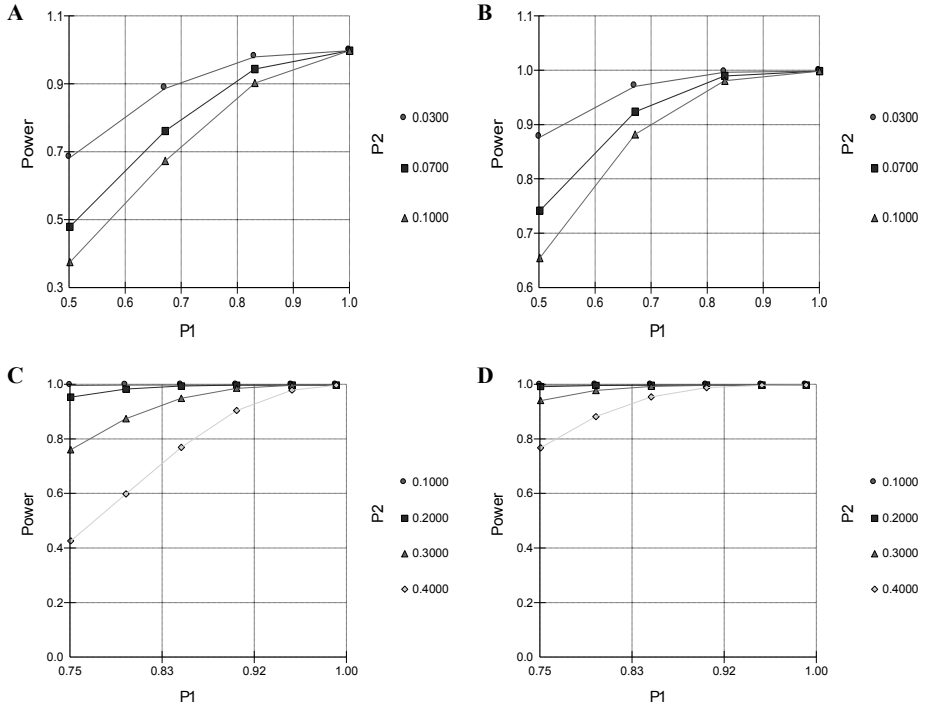

Figure 2. Results of the power analyses. The power is indicated to detect differences between the non-erosive high-inflammatory ('resistant', n=6) and low-inflammatory (n=29) groups (Panel A and B) and high-erosive low-inflammatory, ('sensitive' n=17) and high-inflammatory (n=74) groups (Panel C and D). Power calculations were done for different levels of significance: α of 0.005 (Panel A, C) and 0.5 (Panel B, D). P1 represents the proportion of patients carrying the recessive genotype or the proportion of patients carrying rare mutations in the resistant or sensitive group and P2 represents these proportions in the other group

factors relevant in protection or progression is to appraise patients with extreme responses on inflammation. The present study reports that RA patients with extreme discordant phenotypes can be identified.

We identified two extreme responses on joint inflammation. The 'resistant' phenotype, characterized by the absence of erosive damage despite high cumulative levels of inflammation throughout the studied period, and the 'sensitive' phenotype, characterized by the lowest cumulative inflammation but the highest levels of erosiveness. Considering the 'sensitive' phenotype, a question is whether physical examination on swollen joints was sensitive enough to detect joint inflammation. It is possible that subclinical inflammation was present.[15] However, even in this case, these patients have an extreme sensitive response to subtle local inflammation.

We did not intend to evaluate whether inflammation is associated with joint destruction; this has been studied before on joint level.[16] Moreover, in the present study analyses were performed on patient level to identify patients with extreme responses to inflammation. Because of this aim,

we did not perform analyses on joint level, as this would have resulted in 32 comparisons per patient.

The ultimate question is what processes underlie these extreme phenotypes. Genetic factors may account for an individual's degree of sensitivity to inflammation. It was observed that the power of future genetic studies on rare genetic variants using the number of patients identified is sufficient.

The present study has several limitations. First, the AUC of the SJC was determined using yearly measurements. This may lead to bias since the number of swollen joints at one time-point may not reflect the average number of swollen joints during a year. To prevent misclassification, the medical files of all patients in the high or low inflammatory groups were studied to verify whether the classification fitted with clinical evaluations at time points in between yearly visits. Second, treatment was not taken into consideration. The variety of medications used made adjusting for treatment challenging. It is generally presumed that anti-rheumatic treatment suppresses the level of inflammation. This does not hamper the subject of the present study, which concerns the degree of joint damage in response to inflammation. In case treatment was prescribed that directly affected bone destruction, joint damage may be more diminished than would be the result of suppressing inflammation only. At present, to our knowledge, the only anti-rheumatic treatments that may reduce bone damage to a higher extend than suppressing inflammation are the TNFα inhibitors.[6-9] None of the 6 'resistant' patients were treated with anti-TNF. A third issue is that we studied the SJC and not the level of acute phase reactants or the disease activity score (DAS). We did not study the DAS as it is a composite measure. Pain may increase the DAS also in the absence of synovitis and, vice versa, it is known that a low DAS can be achieved in the presence of inflamed joints. We also chose not to use the CRP as this is a systemic inflammatory-marker, rather than a reflection of local inflammation. A recent study on data from five randomized trials also showed that joint swelling rather than CRP contributes to joint damage.[17]

In conclusion, RA patients with extreme responses in joint destruction to local inflammation are infrequent but prevailing. Further studies in these patients may elucidate mechanisms contributing to the coupling between inflammation and destruction of joints in RA.

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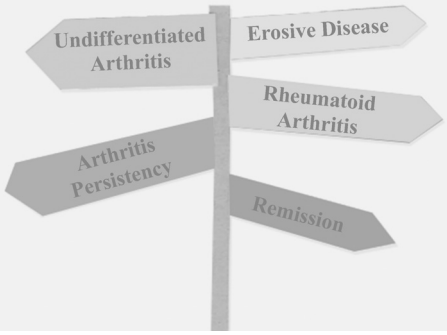
PART VI



Discriminative ability in (development of RA and) outcome of RA

CHAPTER 13

Classification of rheumatoid arthritis: comparison of the 1987 ACR and 2010 ACR/EULAR criteria

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ABSTRACT

Objective

New criteria to classify RA have been derived in order to increase the specificity and sensitivity for early RA compared to the 1987 ACR-criteria. This study evaluated differences in classification between the 1987 ACR-criteria and 2010 ACR/EULAR-criteria in an early arthritis cohort and determined the test characteristics of the 2010 ACR/EULAR-criteria.

Methods

2258 early arthritis patients included in the Leiden EAC cohort were studied. Fulfilment of the 1987- and 2010-criteria for RA was determined at baseline. The diagnosis at 1 year was assessed. The sensitivity and specificity of the 2010-criteria were determined using the following outcome measures: initiation of methotrexate-therapy or any DMARD-therapy during the first year of follow-up and having persistent arthritis during 5 years of follow-up.

Results

At first presentation, 1099 patients fulfilled the 2010-criteria and 726 patients the 1987-criteria for RA. 82 of the 726 patients fulfilling the 1987-criteria did not fulfill the 2010-criteria. 68% of the patients that fulfilled the 1987-criteria during the first year of the disease but not at baseline, did fulfill the 2010-criteria at baseline. The 2010 classification also led in 18% to a revoked classification at year 1. The sensitivity and the specificity were 0.84 and 0.60 with methotrexate therapy as outcome and 0.74 and 0.74 with DMARD therapy as outcome. With arthritis persistency as outcome, the sensitivity and specificity were 0.71 and 0.65.

Conclusion

Compared to the 1987-criteria, the 2010-criteria classify more patients with RA and at an earlier phase. The discriminative ability of the 2010 criteria is acceptable.

INTRODUCTION

During the last decades the focus of the management of RA has shifted to the early phase of the disease. This change was fuelled by studies showing that early achievement of low disease activity states is beneficial for the further course of RA. These studies raised the awareness on the importance of treating early and pointed to the relevance of early recognition of RA. From this perspective, the 1987 ACR criteria for RA[1] have been criticized as they are not equipped to diagnose RA early. This is not surprising as they have been developed in order to define homogeneous patient groups for research purposes and therefore were based on patients with average disease duration of 7 years.

In order to be able to identify early RA patients for clinical trials and other studies new classification criteria for RA have been derived by a task force of experts from both the EULAR and ACR.[2] The main purpose of these 2010 criteria is to achieve an increased sensitivity and specificity for RA in an early phase.

At present the diagnostic and discriminative abilities of these new criteria are not known. In addition it is unclear how the 2010 criteria behave in relation to the 1987 ACR criteria. This is especially relevant because the Working group that developed the 2010 criteria stressed in their discussion that the patients fulfilling the 2010 criteria are probably less homogeneous and that therefore in clinical trials researchers should document both the proportions of study subjects that fulfill the previous (1987) and new RA classification criteria to enable comparisons. Moreover, the working group warned that the 2010 criteria may probably increase heterogeneity by including different phenotypes, thereby making basic science studies more difficult. Therefore, the present study evaluated the following questions: What proportion of early arthritis patients that do not fulfill the 1987 criteria can according to the 2010 criteria be classified as RA? Do all early arthritis patients that fulfill the 1987 criteria fulfill the 2010 criteria as well? Do RA patients indeed fulfill the 2010 criteria at an earlier point in time than the 1987 criteria? In addition, the sensitivity and specificity of the 2010 criteria for RA were assessed; for this analysis three outcome measures were studied: initiation of methotrexate (MTX), initiation of any disease-modifying-antirheumatic-drug (DMARD) and having persistent arthritis over a 5 years follow-up period.

PATIENTS AND METHODS

Patients

The early arthritis patients studied are from the Leiden Early Arthritis Clinic (EAC) cohort, a population-based prospective cohort that started in 1993.[3] Inclusion took place when arthritis was confirmed at physical examination and symptom duration was <2 years. The inclusion criteria had not changed over time. At the first visit, patients and rheumatologists completed questionnaires, physical examination was performed, radiographs were taken and blood was taken for determination of amongst other C-reactive protein (CRP), erythrocyte sedimenta-

tion rate (ESR), IgM-rheumatoid factor (RF) and ACPA (anti-CCP2, Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). Follow-up visits were performed yearly. Written informed consent was obtained from all participants. The study was approved by the local Medical Ethical Committee.

2258 early arthritis patients with at least one year of follow-up were included between 1993 and February 2009. Of these, 1632 were formerly classified as RA (1987 ACR criteria) or undifferentiated arthritis. The remaining 626 early arthritis patients were classified with other diagnoses. The treatment of patients with RA differed; hydroxychloroquine, penicillamine or suphasalazine were the initial DMARDs in the '90s, methotrexate was the initial DMARD since 1999.[4] Patients that were classified for other diagnoses than RA were treated accordingly. The treatment of patients that were undifferentiated was not protocollized.

Application of the 2010 criteria

The 2010 ACR/EULAR criteria were applied as described by Aletaha et al.[2] We used the 66-swollen joint count and 68-tender joint count. According to the guideline, the distal phalangeal joints, 1st carpo-metacarpal joint and 1st metatarso-phalangeal joints were excluded from assessment. Involvement of interphalangeal joints of the feet was considered as small joint involvement. The reference value for RF positivity in our cohort is 5, therefore a level ≥15 was considered high positive. Similarly, the reference value for anti-CCP2 positivity is 25 AU in our cohort and a level of ≥75 AU was considered high positive. An abnormal CRP was according to the reference value defined as ≥10 mg/l, and an abnormal ESR was ≥25 mm/hr for females and ≥15 mm/hr for males. In the new criteria it is stated that presence of a significant erosion is *prima facie* evidence of RA which precludes the need for applying other criteria. However, it is not yet agreed on what size, number or site of erosions is necessary to define erosiveness. Because of this uncertainty, we initially did not consider radiological information when applying the 2010 ACR/EULAR criteria. Afterwards analyses were repeated when patients with a SHS erosionscore ≥2 were classified as having RA as well, irrespective of fulfilling any of the other criteria. In addition, the effect of evaluating 44 or 28 instead of 66/68 joints was assessed.

Analysis

The following analyses were done. First, baseline characteristics of all 2258 early arthritis patients were studied to define the proportions of early arthritis patients that were classified as RA according to the 2010 criteria and the 1987 ACR-criteria. It was assessed whether patients that were diagnosed with RA using the 1987 ACR criteria fulfilled the 2010 criteria as well.

In order to determine whether the 2010 criteria are indeed fulfilled in an earlier stage than the 1987 criteria, patients that did fulfill the 1987 criteria during the first year of disease but not at first presentation were studied (n=297). It was determined how many of these patients already fulfilled the 2010 ACR criteria at baseline, and thus were indeed recognized in a more early phase by the 2010 criteria.

It was also evaluated whether the 2010 criteria would yield "false-positive" classifications. To this end, patients that at baseline fulfilled the 2010 ACR criteria were studied for their diagnosis after one year to determine whether they were classified differently at that time-point.

Finally, the sensitivity and specificity of the 2010 criteria were determined and the area under the receiver operator characteristic curve (AUC) assessed in the patients that were formerly classified as RA or undifferentiated arthritis (n=1632). Patients that received DMARD treatment in a randomized trial were not studied, leaving 1404 regularly treated patients for evaluation. Three outcome measures were used. First, initiation of methotrexate therapy within the first year of follow-up, the same outcome measure as used for the derivation of the 2010 criteria. Since methotrexate was not the anchor drug before 1999, initiation of any DMARD within the first year was studied as well. Thirdly, in the subgroup of patients that achieved 5 years of follow-up (n=790), arthritis persistency was assessed and defined by the absence of a sustained DMARD-free remission. Patients were defined as being in remission if DMARD therapy could be discontinued and no synovitis was detected for at least one year.[5] Analyses were done using SPSS (version 17.0).

RESULTS

The baseline characteristics of all early arthritis patients are presented in Table 1. The characteristics of the subset of patients that at baseline were classified as RA according to the 1987 and 2010 criteria are presented as well.

Agreement in classification

At baseline, 1099 out of 2258 early arthritis patients fulfilled the 2010 criteria for RA. 726 patients fulfilled the 1987 ACR criteria for RA. From these 726 patients, 644 (88.7%) also fulfilled the 2010 criteria whereas 82 (11.3%) patients did not fulfill the 2010 criteria. From the 1099 patients that fulfilled the 2010 criteria, 455 patients did not fulfill the 1987 criteria (Table 2A). From the 1099 patients that fulfilled the 2010 criteria, 455 patients did not fulfill the 1987 criteria (Table 2A). The agreement in classification criteria was not different when patients included before of after 1999 were studied separately (data not shown). Characteristics of the patients that fulfilled both the 1987 and 2010 criteria and that fulfilled the 1987 but not the 2010 criteria are presented in Supplementary Table 1.

Baseline classification in relation to the disease course

297 patients fulfilled the 1987 ACR criteria during the first year, but not at baseline. From these, 202 (68.0%) did fulfill the 2010 criteria at baseline, indicating that the 2010 criteria indeed classify RA patients in an earlier phase of the disease.

The 1099 early arthritis patients that fulfilled the 2010 ACR criteria at baseline were studied for their diagnosis at year 1. In 194 cases patients were classified differently at that time-point. Study-

Table 1. Baseline characteristics of all early arthritis patients, the subset of early arthritis patients that fulfilled the 1987 ACR criteria and the subset that fulfilled the 2010 ACR/EULAR criteria at first presentation

Characteristics	All early arthritis pts (n=2258)	1987 RA (n=726)	2010 RA[$] (n=1099)
Age at inclusion (yrs), mean (SD)	51.9 (17.2)	57.4 (16.3)	56.1 (16.4)
Female, N (%)	1340 (59.3)	470 (64.7)	718(65.3)
Symptom duration at first presentation, weeks, mean (SD)[‡]	25.9 (41.6)	31.6 (36.3)	29.8 (43.4)
< 6 weeks, N (%)[$]	*436 (21.4)*	*0 (0)[†]*	*98 (9.6)*
≥ 6 weeks, N (%)[$]	*1602 (78.6)*	*726 (100)[†]*	*925 (90.4)*
66 Swollen joint count, mean (SD)	6.5 (6.8)	11.6 (7.3)	10.3 (7.7)
1 medium-large joint, N (%)[$]	*253 (11.2)*	*0 (0)*	*0 (0)*
2-10 medium-large joints, N (%)[$]	*142 (6.3)*	*0 (0)*	*3 (0.3)*
1-3 small joints, N (%)[$]	*532 (23.6)*	*48 (6.6)*	*106 (9.6)*
4-10 small joints, N (%)[$]	*560 (24.8)*	*206 (28.4)*	*236 (21.5)*
> 10 joints, N (%)[$]	*771 (34.1)*	*472 (65.0)*	*754 (68.6)*
ESR (mm/hr), mean (SD)[‡]	33.2 (28.1)	40.3 (28.2)	38.3 (28.0)
CRP (mg/l), mean (SD)[‡]	27.1 (28.1)	28.2 (35.2)	28.3 (35.1)
Normal CRP and ESR, N (%)[$]	*747 (33.1)*	*147 (20.2)*	*251 (22.8)*
Abnormal CRP or ESR, N (%)[$]	*1511 (66.9)*	*579 (79.8)*	*848 (77.2)*
RF positive, N (%)[‡]	671 (30.1)	399 (55.0)	601 (55.3)
Anti-CCP2-positive, N (%)[‡]	506 (29.7)	323 (51.4)	472 (52.2)
Negative RF and ACPA, N (%)[$]	*1484 (65.7)*	*285 (39.3)*	*412 (37.5)*
Low positive RF or ACPA, N (%)[$]	*203 (9.0)*	*78 (10.7)*	*145 (13.2)*
High positive RF or ACPA, N (%)[$]	*571 (25.3)*	*363 (50.0)*	*542 (49.3)*
Erosive, N (%)	590 (26.1)	392 (54.0)	467 (42.5)

[$]applied without considering data on erosiveness at baseline. [‡]Data missing for analysis (n): symptom duration (220); ESR (15); CRP (219); RF (32); anti-CCP2 (553). [†]According to the 1987 ACR criteria, 4/7 criteria have to exist for >6 weeks to be a valid criterion. [$]Subdivision of criteria according to the score based algorithm from the 2010 ACR/EULAR criteria

ing the medical records of these patients confirmed that these patients clinically had evidently another diagnosis than RA. The diagnoses of these patients were: psoriatic arthritis (n=46), inflammatory osteoarthritis (n=28), reactive arthritis (n=20), RS3PE (n=17), sarcoidosis (n=15), (pseudo)gout (n=15), para-malignant arthritis (n=6), spondylarthropathy (n=6), SLE (n=10), MCTD (3), other systemic disorders (n=21) and other diagnoses (n=7). These patients concern 17.7% of the total population of patients fulfilling the 2010 ACR criteria and 27.7% of the patients that at baseline did fulfill the 2010 criteria but not the 1987 criteria.

In the description of the 2010 criteria is stated that these should be applied only in case no other diagnosis can be established. Thus this means that in case a patient can be classified with two diagnoses, the other diagnosis prevails. Therefore we repeated the analysis presented above in the patients that were formerly classified as RA or undifferentiated arthritis (n=1632). Of

Table 2. Classification according to the 1987 and 2010 criteria for RA without A) and with B) including radiologic information on erosiveness when applying the 2010 ACR/EULAR criteria

		(A) 2010 ACR/EULAR Classification Criteria[§]		(B) 2010 ACR/EULAR Classification Criteria[†]		
		RA at baseline	no RA baseline	RA at baseline	no RA baseline	*Total*
1987 ACR Classification Criteria	RA at baseline	644	82	678	48	*726*
	no RA at baseline	455	1077	544	988	*1532*
	Total	*1099*	*1159*	*1222*	*1036*	*2258*

[§]application of the 2010 ACR/EULAR criteria without the use of radiologic information on erosiveness. [†]application of the 2010 ACR/EULAR criteria with the use of radiologic information on erosiveness and defining erosiveness as a total SHS erosion score ≥2. Criteria were applied using data on 66/68 joints for swelling/tenderness

these, 939 patients fulfilled the 2010 ACR criteria at baseline and were studied for their diagnosis at year 1. In 88 cases patients were classified differently at that time-point.

Studying the medical records of these patients confirmed that these patients clinically had evidently another diagnosis than RA. The diagnoses of these patients then were: psoriatic arthritis (n=20), inflammatory osteoarthritis (n=13), reactive arthritis (n=7), RS3PE (n=7), (pseudo)gout (n=7), SLE (n=6), para-malignant arthritis (n=4), spondylarthropathy (n=4), sarcoidosis (n=3), MCTD (2), other systemic disorders (n=12) and other diagnoses (n=3). These patients concern 9.4% of the 939 patients that fulfilled the 2010 ACR criteria and 14.1% of the patients that at baseline did fulfill the 2010 criteria but not the 1987 criteria.

Test characteristics of the 2010 criteria

When using initiation of methotrexate-therapy within the first year as outcome the sensitivity and specificity of the 2010 criteria were 0.84 and 0.60. With initiation of any DMARD-therapy within the first year as outcome the sensitivity and specificity were 0.74 and 0.74. The AUCs when using these two outcomes were 0.72 and 0.74 respectively. The third outcome measure was arthritis persistency over 5 years. Here the sensitivity of the 2010 criteria was 0.71, the specificity 0.65 and the AUC 0.65 (Table 3A).

Value of baseline erosiveness

It is unclear what number of erosions in early arthritis patients is specific for early RA. In the evaluation of the consequences of considering erosiveness, here defined as total SHS erosion score ≥2, we observed that 1222 patients were at baseline classified as RA according to the 2010 criteria. Thus when including erosiveness in the evaluation, 123 (5.4%) early arthritis patients were additionally classified as RA. The analyses on the agreement in classification and on the test

Table 3. Test characteristics of the 1987 and 2010 criteria using three outcome measures: methotrexate initiation during the first year, initiation of any DMARD during the first year and having disease persistency over 5 years. Analyses were done without (A) and with (B) including radiologic information on erosiveness when applying the 2010 ACR/EULAR criteria

	Outcome Measure								
	MTX-initiation			DMARD-initiation			5-year Persistency		
Criteria Set	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC
1987 ACR Classification Criteria	0.61	0.74	0.67	0.54	0.87	0.71	0.53	0.75	0.61
(A) 2010 ACR/EULAR Classification Criteria[§]	0.84	0.60	0.72	0.74	0.74	0.74	0.71	0.65	0.65
(B) 2010 ACR/EULAR Classification Criteria[†]	0.88	0.54	0.71	0.79	0.68	0.79	0.77	0.56	0.65

[§]application of the 2010 ACR/EULAR criteria without the use of radiologic information on erosiveness. [†]application of the 2010 ACR/EULAR criteria with the use of radiologic information on erosiveness and defining erosiveness as a total SHS erosion score ≥2

characteristics were repeated (Table 2B and 3B), but the results were not substantially different compared to the results when radiological information was disregarded.

Effect of number of assessed joints

We used a 66/68 count for swollen and tender joints. In clinical practice 44 or 28 joints may be evaluated more frequently. In order to determine whether this would results in different classification, the 2010 criteria were applied with the 44 and 28 joint counts. The numbers of patients classified as RA were then 1082 and 940 respectively, instead of 1099 (Supplementary Table 2). The test characteristics were fairly comparable when a lower number of joints was considered (Supplementary Table 3).

DISCUSSION

The present study compared classification of RA using the 1987 and 2010 criteria in a large early arthritis cohort. It was observed that the 2010 criteria classified more patients with RA than the 1987 criteria and that 11.3% of the patients with RA according to the 1987 criteria were not classified as RA according to the 2010 criteria. A large proportion of the early arthritis patients that developed RA according to the 1987 criteria during the disease course could at first presentation be classified as RA according to the 2010 criteria. This denotes that the 2010 criteria have come up to the demand of classifying RA in an earlier phase of the disease than the 1987 criteria. The 2010 classification also led in 18% (or 9.4% dependent on the studied population) to a revoked diagnosis in a later phase, substantiating the concerns with regard to increase in heterogeneity by use of the 2010 criteria. Compared to the 1987 criteria, the sensitivity of the 2010 criteria was higher but the specificity lower.

In this study several choices were made when applying the 2010 ACR/EULAR criteria. Initially we left out information on hand and feet radiographs as a clear description of erosive disease resembling RA was not provided by Aletaha et al.[2] Afterwards we repeated analyses defining total SHS erosionscore ≥2 as erosiveness. Fairly similar observations were done. This may suggest that evaluating radiographs in this early phase is not highly relevant for classification of RA. A moderate predictive ability of the number of erosive joints in early arthritis patients for RA development has been described recently.[6]

We used a 66/68 count for swollen and tender joints. In clinical practice 44 or 28 joint counts are frequently used. To evaluate the effect of assessing different numbers of joints we repeated the analyses with these joint counts. The number of patients classified with RA decreased but the test characteristics were only marginally affected.

This study has some limitations. First of all it is based on one inception cohort and more studies are needed to establish the sensitivity and specificity of the new criteria. A complicating factor is that it is somewhat ambiguous what outcome measure to take as the gold standard for RA. This has been subject to discussion within the working group that derived the 2010 criteria

and usage of methotrexate was chosen. This outcome may not be appropriate when studying older cohorts. For example in the 1990's it was not common practice to start methotrexate early in a patient with arthritis of recent onset that did not fulfill the 1987 criteria. For this reason we chose any DMARD-therapy instead of methotrexate-therapy as outcome. Additionally we chose a second outcome (arthritis persistency over 5 years) for verification. However, differences in the outcome measure may yield variations in observed test characteristics.

The three outcome measures used here (initiation of methotrexate or any DMARD or arthritis persistency) all contain risk of misclassification as these can also be fulfilled in case of other diagnoses, for example in psoriatic arthritis. Psoriatic arthritis was also the most frequent cause of a revoked classification at year 1.

Another consideration is that 213 early arthritis patients included in the EAC after 2000 were used in the derivation phase of the 2010 criteria. In the present study we evaluated a considerable larger number of patients, as well as two outcome measures additional to methotrexate usage. In order to see whether this subset of patients affected the results, analyses were repeated excluding the 213 patients. This did not yield substantially different results (data not shown). Nonetheless, validation of the 2010 criteria in other cohorts is required as well.

Given the emerging evidence on a "window of opportunity",[7] pointing to the need to treat as early as possible, the question is what method serves best to identify individual patients in an early phase of RA. The authors of the 2010 criteria underline that the new classification criteria were not developed as a diagnostic tool and that a separate body of work is needed to develop such tools.[2] Prediction rules aiming at early diagnosis have been developed and validated.[8,9] The question what method is best to identify early RA on the individual patient level is still open and a subject for future studies.

In conclusion, the 2010 criteria for RA classify more patients with RA and do so in an earlier phase. The discriminative ability of the 2010 ACR/EULAR criteria is reasonable, indicating that these criteria perform well to classify early RA.

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Supplementary Table 1. Baseline characteristics of all early arthritis patients, the subsets of early arthritis patients that fulfilled the 1987 ACR criteria but not the 2010 criteria and that fulfilled both the 1987 and the 2010 ACR/EULAR criteria at first presentation. Data are presented without and with assessing radiologic information on erosiveness when applying the 2010 ACR/EULAR criteria

Characteristics	All early arthritis pts (n=2258)	1987+/2010- (n=82)[§]	1987+/2010+ (n=644)[§]	1987+/2010- (n=48)[†]	1987+/2010+ (n=678)[†]
Age at inclusion (yrs), mean (SD)	51.9 (17.2)	55.8 (17.4)	57.6 (16.2)	53.3 (18.8)	57.7 (16.1)
Female, N (%)	1340 (59.3)	51 (62.2)	419 (65.1)	29 (60.4)	441 (65.0)
Symptom duration at first presentation in weeks, mean (SD)	25.9 (41.6)	25.4 (23.1)	32.4 (37.6)	26.3 (24.6)	32.0 (37.0)
< 6 weeks, N (%)	*436 (21.4)*	*0 (0)[†]*	*0 (0)*	*0 (0)*	*0 (0)*
≥ 6 weeks, N (%)	*1602 (78.6)*	*79 (100)[†]*	*626 (100)*	*47 (100)*	*658 (100)*
66 Swollen joint count, mean (SD)	6.5 (6.8)	5.9 (2.2)	12.3 (7.4)	5.6 (2.1)	12.3 (7.4)
1 medium-large joint, N (%)	*253 (11.2)*	*0 (0)*	*0 (0)*	*0 (0)*	*0 (0)*
2-10 medium-large joints, N (%)	*142 (6.3)*	*0 (0)*	*0 (0)*	*0 (0)*	*0 (0)*
1-3 small joints, N (%)	*532 (23.6)*	*19 (23.2)*	*29 (4.5)*	*10 (20.8)*	*38 (5.6)*
4-10 small joints, N (%)	*560 (24.8)*	*63 (76.8)*	*143 (22.2)*	*38 (79.2)*	*168 (24.8)*
> 10 joints, N (%)	*771 (34.1)*	*0 (0)*	*472 (73.3)*	*0 (0)*	*472 (69.9)*
ESR (mm/hr), mean (SD)	33.2 (28.1)	32.9 (23.9)	41.3 (28.5)	32.3 (26.7)	40.8 (28.2)
CRP (mg/l), mean (SD)	27.1 (28.1)	27.5 (29.5)	31.6 (35.9)	23.3 (29.5)	31.7 (35.5)
Normal CRP and ESR, N (%)	*747 (33.1)*	*23 (28.0)*	*124 (19.3)*	*16 (33.3)*	*275 (40.9)*
Abnormal CRP or ESR, N (%)	*1511 (66.9)*	*72.0 (72.0)*	*520 (80.7)*	*32 (66.7)*	*398 (59.1)*
RF positive, N (%)	671 (30.1)	1 (1.2)	399 (62.4)	1 (2.1)	399 (62.4)
Anti-CCP2-positive, N (%)	506 (29.7)	2 (3.0)	321 (57.1)	2 (5.7)	321 (57.1)
Negative RF and ACPA, N (%)	*1484 (65.7)*	*80 (97.6)*	*205 (31.8)*	*45 (93.8)*	*240 (35.4)*
Low positive RF or ACPA, N (%)	*203 (9.0)*	*0 (0.0)*	*76 (11.8)*	*0 (0)*	*75(11.1)*
High positive RF or ACPA, N (%)	*571 (25.3)*	*3 (3.7)*	*363 (56.4)*	*3 (6.3)*	*363 (53.5)*
Erosive, N (%)	590 (26.1)	34 (41.5)	356 (55.3)	0 (0)	390 (57.5)

[§]application of the 2010 ACR/EULAR criteria without the use of radiologic information on erosiveness. [†]application of the 2010 ACR/EULAR criteria with the use of radiologic information on erosiveness and defining erosiveness as a total SHS erosion score ≥2

Supplementary Table 2. Classification according to the 1987 and 2010 criteria for RA without (A) and with (B) including radiologic information on erosiveness when applying the 2010 ACR/EULAR criteria

			(A) 2010 ACR/EULAR Classification Criteria[§]		(B) 2010 ACR/EULAR Classification Criteria[†]		
			RA at baseline	no RA baseline	RA at baseline	no RA baseline	*Total*
Assessing 44 joints[‡]	1987 ACR classification Criteria	RA at baseline	639	87	677	49	*726*
		no RA at baseline	443	1089	533	999	*1532*
		Total	*1082*	*1176*	*1210*	*1048*	*2258*
Assessing 28 joints[$]	1987 ACR classification Criteria	RA at baseline	603	123	653	73	*726*
		no RA at baseline	337	1195	443	1089	*1532*
		Total	*940*	*1318*	*1096*	*1162*	*2258*

[‡]Criteria were applied using the 44 joint counts data. [$]Criteria were applied using the 28-swollen joint counts. [§]application of the 2010 ACR/EULAR criteria without the use of radiologic information on erosiveness [†]application of the 2010 ACR/EULAR criteria with the use of radiologic information on erosiveness and defining erosiveness as a total SHS erosion score ≥2

Supplementary Table 3. Test characteristics of the 1987 and 2010 criteria using methotrexate initiation during the first year, initiation of any DMARD during the first year and having disease persistency over 5 years as outcome measures. Analyses were done without (A) and with (B) including radiologic information on erosiveness when applying the 2010 ACR/EULAR criteria

		Outcome Measure								
		MTX-initiation			DMARD-initiation			Persistency over 5 years		
	Criteria Set	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC
Assessing 44 joints[‡]	1987 ACR Classification Criteria	0.61	0.74	0.67	0.54	0.87	0.71	0.53	0.75	0.61
	(A) 2010 ACR/EULAR Classification Criteria[§]	0.83	0.60	0.71	0.73	0.74	0.74	0.71	0.65	0.65
	(B) 2010 ACR/EULAR Classification Criteria[†]	0.87	0.54	0.71	0.79	0.68	0.74	0.77	0.56	0.65
Assessing 28 joints[§]	1987 ACR Classification Criteria	0.61	0.74	0.67	0.54	0.87	0.71	0.53	0.75	0.61
	A) 2010 ACR/EULAR Classification Criteria[§]	0.83	0.60	0.71	0.73	0.74	0.72	0.65	0.69	0.64
	B) 2010 ACR/EULAR Classification Criteria[†]	0.87	0.54	0.71	0.79	0.68	0.73	0.74	0.59	0.64

[‡]Criteria were applied using the 44-joint count. [§]Criteria were applied using the 28- joint count. [§]application of the 2010 ACR/EULAR criteria without the use of radiologic information on erosiveness. [†]application of the 2010 ACR/EULAR criteria with the use of radiologic information on erosiveness and defining erosiveness as a total SHS erosion score ≥2

CHAPTER 14

Predicting arthritis outcomes - what can be learned from the Leiden Early Arthritis Clinic?

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ABSTRACT

Introduction

In order to allow personalized medicine, adequate prediction of the disease outcome is required. In early undifferentiated arthritis (UA) prediction of the development of rheumatoid arthritis (RA) is crucial and in case of RA predicting the severity of the disease course may guide individualized treatment decisions.

Methods

570 UA patients and 676 RA patients included in the Leiden Early Arthritis Clinic cohort were studied for baseline characteristics. The disease outcomes studied were fulfillment of the 1987 ACR RA criteria and arthritis persistency in UA patients and the rate of radiological joint destruction and achieving sustained DMARD-free remission in RA patients.

Results

Predictive factors for fulfillment of the 1987 ACR RA criteria and for persistent arthritis in UA were largely similar. Risk factors for a severe rate of joint destruction were: (p-value) older age (<0.001); male gender (<0.001); longer symptom duration at first visit (0.048), involvement of lower extremities (<0.001); BMI (<0.001); high acute phase reactants, presence of IgM-RF (<0.001); anti-CCP2 antibodies (<0.001); anti-MCV antibodies (<0.001) and HLA-SE alleles (0.001). A high BMI was associated with a lower rate of joint destruction but with a higher risk on disease persistency. The proportion of variance in joint destruction explained was 32%.

Conclusion

Predictors for RA development, previously used to develop a prediction rule in UA patients, are largely similar to predictors for arthritis persistency. Only part of the level of joint destruction in RA is explained by currently known risk factors. New factors need to be identified in order to guide pharmaceutical intervention at the level of individual RA patients.

INTRODUCTION

The outcome of early arthritis patients is highly variable. Approximately only one-third of the patients with a recent-onset undifferentiated arthritis (UA) progresses towards rheumatoid arthritis (RA). The severity of the progression of joint destruction in RA is highly variable as well, as only a minority will become severely destructed. In order to achieve individualized treatment decision making, the severity of the disease outcome needs to be estimated adequately. This is particularly relevant since it is widely acknowledged that early initiation of treatment of RA is effective in diminishing the level of joint destruction and disability.[1-3] Fewer studies are performed on the effects of early intervention in recent-onset UA, but available data suggest that early treatment strategies hamper progression in UA as well.[4-6] Potent treatment strategies such as targeted therapies are generally not started in an early phase because of the risk of overtreatment. However, when the individuals who will have an unfavorable disease outcome can be identified at first presentation, the risk on overtreatment and undertreatment can be balanced, resulting in a personalized pharmaceutical regimen.

Observational studies of unselected patients are most appropriate to identify risk factors for a certain disease course. Following patients with and without risk factors allows direct assessments of absolute risks on a disease outcome. The Leiden Early Arthritis Clinic cohort is a population based inception cohort including early arthritis patients since 1993. Patients are being followed as long as they are seen at the rheumatologist and follow-up ends in case patients are discharged because of having a sustained DMARD-free remission or when patients die. During the past years several risk factors for a mild or progressive disease course, both in UA and RA, have been identified.

The present manuscript in this themed issue on Registries in Rheumatologic conditions reviews to what extend the disease outcome in early UA and early RA can be predicted, using data from the Leiden Early Arthritis Clinic cohort. The two disease outcomes studied in UA are fulfilling the 1987 ACR criteria for RA and having persistent arthritis. The disease outcomes studied in early RA patients are the progression in joint destruction over time and disease persistency. These evaluations allow comparison of risk factors for joint destruction and RA persistency. Since it is thus far unclear to what extent the processes underlying joint destruction are similar to the processes that mediate disease persistency, evaluation of overlapping and dissimilar risk factors may increase understanding and the subsequent elucidation of the underlying biological pathways leading to these phenotypic characteristics. Finally, the fraction of explained variance of progression in joint destruction by the currently known risk factors is determined to asses how complete our current understanding is.

PATIENTS AND METHODS

Design of Leiden Early Arthritis Clinic

This Leiden EAC is a population-based prospective cohort that was started in 1993 in order to detect and treat inflammatory disorders early in the disease state, especially early RA. In order to obtain early referrals by general practitioners (GPs), a campaign was started among GPs to refer patients with suspected arthritis as soon as possible to the rheumatology department of the Leiden University Medical Center. This is the only centre for rheumatic diseases in a semi-rural area with more than 400,000 inhabitants. Patients are seen within 2 weeks. Inclusion took place when arthritis was confirmed at physical examination and symptom duration was less than two years. Written informed consent was obtained from all participants. The study was approved by the local Medical Ethical Committee. At the first visit, the rheumatologist completed a questionnaire regarding the presenting symptoms, as reported by the patient: type, localization and distribution of initial joint symptoms, symptom duration, and course of the initial symptoms. The patient's smoking history and family history were assessed. Patients rated morning stiffness on a visual analogue scale (VAS; range 0-100 mm); the duration of morning stiffness was also assessed. The Health Assessment Questionnaire (HAQ) was used to provide an index of disability. A 66-joint count for swollen joints (SJC) was performed. Blood samples were taken for routine diagnostic laboratory screening (including C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR) and immunoglobulin (Ig)M-rheumatoid factor (RF)) and stored to determine other serum markers (amongst others antibodies against citrullinated peptide antibodies) at a later time. Blood samples were taken for DNA extraction as well. Follow-up visits with standard clinical assessments (including a SJC and a HAQ) were performed 3 months after the first presentation and yearly thereafter. Radiographs of the hands and feet were taken at baseline and yearly thereafter. Two weeks after inclusion, when results of laboratory investigations and radiography were known, patients that had a form of arthritis that could not be classified according to American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria were documented as having UA. The diagnosis RA was established in case patients fulfilled the 1987 ACR criteria for RA. The initial treatment of RA patients had changed in time and differed according to the inclusion period.[7] Patients included between 1993 and 1995 were initially treated with analgesics and were subsequently treated with hydroxychloroquine or sulfasalazine if they had persistent active disease. Between 1996 and 1998, patients who were included were promptly treated with chloroquine or sulfasalazine, while after 1998, the initial treatment strategy consisted of either methotrexate or sulfasalazine.[7] Treatment of UA patients was not protocolized.

Definition of outcome measures

Patients with UA were assessed on two outcomes. First, after one year of follow up, the fulfillment of the 1987 ACR criteria for RA was evaluated. As previously described, 31% of UA patients pro-

gressed to RA during 1 year of follow-up. The majority of the patients (94%) had been followed up for more than 1 year (mean follow-up 8 years, SD 3 years) and 4.4% of UA patients developed RA later than one year after inclusion.[8] The second disease outcome was disease persistency. As a generally accepted definition for persistency is lacking, we defined persistent arthritis as the absence of sustained DMARD-free remission. Sustained remission was diagnosed when patients had no swollen joints for at least one year after cessation of eventual DMARD therapy. The absence of swollen joints had to have been observed by a rheumatologist for at least one year to ensure that remission was not temporary, but rather sustained. When remission was not obtained after 5 years of disease, a patient was classified as having persistent disease in the present study.

The RA patients were studied for the rate of radiological joint destruction and for achieving sustained DMARD-free remission or having persistent RA, also during a five years period of follow-up. In order to study the progression rate in a sensitive way all serial radiographs were scored by one experienced reader (MvdL) according to the Sharp-van der Heijde method (SHS) in chronological order. Four hundred and nine radiographs belonging to 60 randomly selected RA patients were rescored. The intraclass-observer correlation coefficient was 0.91 for all scored radiographs, and 0.97 for the radiographic progression rate. The means (±SEM) at the subsequent time points were 9.15 (0.43) at baseline; 15.65 (0.72) at one year follow up; 20.0 (0.93) at two years; 24.79 (1.36) at three years; 34.83 (2.14) at four years and 34.8 (2.14) at five years of follow-up. Persistent RA was defined as the absence of a sustained DMARD-free remission. A sustained DMARD free remission in RA was defined as the absence of swollen joints for at least one year after cessation of DMARDs and classification as DMARD-free remission by the rheumatologist. To ensure that remission was not temporary but rather sustained and long-lasting, the absence of swollen joints had to have been observed by a rheumatologist for at least 1 year after discontinuation of DMARD therapy. Corticosteroids were here considered to be equivalent to DMARDs. The majority of patients with disease in remission were discharged from the outpatient clinic at any time, however most patients who achieved remission were followed up longer than the minimum requirement of 1 year; the median time of observation after discontinuation of DMARDs in the absence of swollen joints was 2.5 years. Patients who had a recurrence of their arthritis after discharge could easily return to the Leiden University Medical Center. The frequency of disease relapse was 6%; these patients were included in the persistency group. We observed previously that sustained DMARD-free remission was obtained by 15% of RA patients after a median disease duration of 43 months.[9] Therefore, for the present study, patients that within the first 5 years did not achieve a sustained DMARD-free remission were classified as having persistent RA.

Statistical analysis

Predictors for RA development and arthritis persistency were analyzed univariately with a logistic regression analysis. Since the aim of the present study was to review predictive factors

and not to develop a prediction rule for the outcome of UA, which has been done before,[10] no multivariate regression analysis was performed in UA patients.

Associations between baseline factors and rate of joint destruction were analyzed with a linear multivariate regression model see ref. for detailed description.[7] This was done for each variable separately, but all analyses were adjusted for the applied treatment strategy. In a previous study we showed that the inclusion period is an adequate proxy for the different treatments strategies that were applied over time.[7] The baseline characteristics were tested with an interaction term of a linear function of time. The risk estimate (β) resulting from these analyses reflected the relative difference in slopes between the groups over five years of follow-up. To test for a difference that is not progressive but stable over time, a model without interaction term was fitted; the overall effect of the risk factor then reflected a constant effect in time. This model does not exclude patients in case of missing radiographs and can deal with missingness provided that it is missingness at random.[7] Patients with complete datasets are weighted more heavily in the analysis than patients with missing radiographs.

All factors that were associated with the progression of joint destruction were entered in a multivariate analysis to determine the variance of joint destruction explained by these factors. This variance was defined by comparing the residual variance of the analysis including all risk factors with the residual variance of the analysis including only the adjustment factor for treatment strategy (inclusion period). The proportional reduction of the residual variance was the explained variance of the risk factors analyzed.

P-values <0.05 were considered significant. Since the aim was to review baseline characteristics in relation to the disease outcome, p values were presented without corrections for multiple testing. Analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

Predictors for disease outcome in UA

177 UA patients developed RA (31%). An overview of baseline characteristics associating with RA development and persistency of arthritis is presented in Table 1. Part of the variables predictive for the development of RA was described previously.[10-12] Identified variables associating with the development of RA were patients' characteristics (age, gender, having a positive family history of RA), morning stiffness, inflammatory characteristics (CRP, ESR, number of swollen joints), localization of involved joints, and presence of auto-antibodies (RF, anti-CCP2, and anti-MCV). The environmental factors smoking and BMI were not associated with progression from UA to RA. The acuteness of the start of the complaints was associated with RA development; UA patients with a gradual onset of symptoms had a 1.5 higher odds ratio to develop RA than patients with a subacute symptom onset. A longer duration of symptoms at first presentation was associated with a higher risk on the development of RA as well.

Table 1. Baseline characteristics of patients with undifferentiated arthritis in relation to the outcome measures RA development and persistency of arthritis

		RA development		Arthritis Persistency	
Baseline characteristic	Frequency	OR (95%CI)	P	OR (95%CI)	P
Age, mean (SD), years	60.0 (16.8)	1.03 (1.01-1.04)	<0.001	1.00 (0.99-1.01)	0.90
Female gender, n (%)	329 (57.7)	2.00 (1.35-2.86)	<0.001	1.47 (1.03-2.08)	0.034
Pos. family history for RA, n (%)	135 (23.7)	1.65 (1.11-2.45)	0.013	1.32 (0.87-1.98)	0.20
Chronic symptom vs. (sub)acute, n (%)	244 (42.8)	1.54 (1.11-2.23)	0.010	1.19 (0.84-1.69)	0.34
Symptom duration at fist visit, mean (SD), weeks	23.3 (23.6)	1.012 (1.004-1.019)	0.002	1.011 (1.002-1.019)	0.012
Morning stiffness severity - VAS (0-100), mean (SD)	41.3 (31.1)	1.02 (1.01-1.03)	<0.001	1.00 (1.00-1.01)	0.19
BMI, mean (SD)	26.0 (12.0)	1.03 (0.99-1.08)	0.18	1.07 (1.01-1.13)	0.013
Localization initial joint symptoms					
Small vs. large joints, n (%)	266 (57.5)	2.48 (1.63-3.79)	<0.001	0.95 (0.65-1.40)	0.80
Large & small vs. large joints, n (%)	107 (35.2)	4.18 (2.50-6.97)	<0.001	1.25 (0.76-2.06)	0.38
Upper vs. lower extremities, n (%)	248 (43.5)	2.21 (1.36-3.57)	0.001	1.02 (0.68-1.53)	0.92
Upper & lower vs. lower extremities, n (%)	161 (50.0)	6.07 (3.63-10.10)	<0.001	2.13 (1.31-3.46)	0.002
Symmetric vs. asymmetric, n (%)	265 (46.5)	2.82 (1.98-4.03)	<0.001	1.20 (0.85-1.71)	0.29
Past or present smoker vs. non-smoker, n (%)	271 (48)	1.0 (0.9-1.4)	0.98	0.7 (0.5-1.1)	0.10
SJC, mean (SD)	3.8 (4.0)	1.17 (1.11-1.23)	<0.001	1.07 (1.02-1.13)	0.01
CRP (mg/L), mean (SD)	21.4	1.01 (1.00-1.02)	0.001	1.01 (1.00-1.01)	0.03
ESR (mm/1hr), mean (SD)	29.5 (24.8)	1.02 (1.01-1.02)	<0.001	1.01 (1.00-1.02)	0.003
IgM-RF positive, n (%)	140 (24.6)	5.10 (3.39-7.66)	<0.001	3.55 (2.18-5.76)	<0.001
Anti-CCP2 positive, n (%)	121 (21.2)	8.74 (5.51-13.84)	<0.001	5.97 (3.30-10.78)	<0.001
Anti-MCV positive, n (%)	172 (33.9)	6.48 (4.32-9.71)	<0.001	4.53 (2.87-7.17)	<0.0001
HLA-SE positive, n (%)	309 (55.9)	1.96 (1.36-2.81)	<0.001	1.76 (1.23-2.51)	0.002

Age, BMI, ESR, CRP, SJC, symptom duration at first visit and morning stiffness were analyzed as continuous variables; this means that the presented OR indicates the odds per unit. For instance, an OR of 1.03 for age in relation to the risk on RA development means that per year increase in age, the OR is 1.03. Morning stiffness is displayed in millimetres. From all 570 patients data on RA-development was present, the remission/persistency state could be reliably determined in 538 patients and was not clear recorded in the medical file in 43 cases. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; BMI: Body Mass Index; SJC: swollen joint count; HLA-SE: HLA-DRB1 shared epitope alleles; RF: rheumatoid factor; anti-CCP2: anti-cyclic citrullinated peptide antibody; anti-MCV: anti-modified citrullinated vimentin antibodies

As the outcome measure of fulfilling the 1987 ACR criteria for RA might be subject to discussion (because these criteria were not designed to identify RA in an early phase) and to circular reasoning (because the presence of hand erosions are part of the ACR criteria), we also tested these baseline characteristics in relation to arthritis persistency, defined as the absence of sus-

tained remission. During the five year period of follow up, 210 UA patients achieved remission (39%). The median disease duration till remission was achieved was 17 months (IQR 6.3-37). Factors significantly associated with disease persistency were inflammatory markers (the number of swollen joints, CRP and ESR) and presence of auto-antibodies. Other characteristics such as the distribution of involved joints, the acuteness of the onset of the complaints, and morning stiffness were not predictive for having a persistent form of arthritis.

Predictors for outcome of RA

Baseline characteristics of RA patients associated with the severity of joint destruction over time are presented in Table 2. The strongest association with the rate of joint destruction was seen for presence of anti-cyclic citrullinated peptide 2 (anti-CCP2). Anti-CCP positive RA patients had over the 5 year period a 2.4 times higher progression rate than anti-CCP negative patients. A similar effect was seen for presence of IgM-RF. Higher levels of acute phase reactants at first presentation were also associated with more severe joint damage over time. RA patients whom initial joint symptoms were located at the lower extremities had a higher rate of joint destruction. Interestingly, the severity of morning stiffness at first presentation was not associated with the severity of joint destruction over time. The body mass index (BMI) was inversely correlated with the progression of joint destruction over time. Few genetic factors are convincingly reported to associate with progression of joint destruction. Here we studied the HLA-Shared Epitope (SE) alleles and *CD40*, both are identified risk factors for anti-CCP positive RA only.[11,13] Although presence of the HLA-SE alleles associated with the progression of joint destruction in RA, *CD40* did not reveal such an association in a cohort consisting of both anti-CCP positive and anti-CCP negative RA patients. All the analyses on the rate of joint destruction were adjusted for the treatment strategy applied; this variable was significantly associated with the rate of joint destruction in all performed analyses.

Since it is unclear whether the processes driving joint destruction are the same that drive RA persistency, predictive factors for both outcomes of RA were compared. The proportion of patients that achieved a sustained DMARD-free remission was 0.157, thus 84.3% of the patients were classified as having persistent RA. The median disease duration till remission was 40 months (IQR 25.5-66.5). The factors that were clearly associated with RA persistency were presence of auto-antibodies, the HLA-SE alleles and the duration of symptoms at the first visit. A high BMI was associated with a higher chance on RA persistency. Although the characteristics indicative for the level of inflammation (CRP, ESR, and SJC) were associated with severity of joint destruction, they were not predictive for having a persistent form of RA.

Fraction of variance of progression in joint destruction explained

The total variance of joint destruction at 5 years explained by the baseline characteristics studied was 32%. Subsequently we aimed to study the contribution of the individual risk factors to the explained variance. This was accomplished by calculating the proportion of the effect size of the

Table 2. Baseline characteristics of patients with rheumatoid arthritis in relation to the outcome measures rate of joint destruction and RA persistency

		Rate of joint destruction over 5 years of follow-up		RA Persistency	
Baseline characteristic	Frequency	β (95%CI)	P	OR (95%CI)	P
Age, mean (SD), years	56.4 (15.7)	1.14 (1.11-1.16)	<0.001*	0.99 (0.97-1.00)	0.070
Female gender, n (%)	459 (67.9)	0.74 (0.63-0.86)	<0.001*	0.85 (0.50-1.45)	0.553
Pos family history for RA, n (%)	173 (26.5)	1.079 (0.92-1.27)	0.354	2.27 (1.18-4.36)	0.014
Chronic symptom vs. (sub)acute, n (%)	287 (44.6)	1.10 (0.94-1.27)	0.234	1.55 (0.93-2.59)	0.095
Symptom duration at fist visit, mean (SD), weeks	26.4 (22.4)	1.00 (1.00-1.00)	0.048	1.02 (1.01-1.03)	0.007
Morning stiffness severity - VAS (0-100), mean (SD)	55.2 (28.7)	1.00 (1.00-1.00)	0.874	1.00 (0.99-1.01)	0.827
BMI, mean (SD)	25.8 (3.8)	0.96 (0.94-0.98)	<0.001	1.11 (1.01-1.23)	0.034
Localization initial joint symptoms					
Small vs. large joints, n (%)	356 (75.7)	1.01 (0.83-1.24)	0.923	0.66 (0.34-1.28)	0.216
Large & small vs. large joints, n (%)	177 (60.8)	0.92 (0.74-1.15)	0.470	0.96 (0.45-2.06)	0.911
Upper vs. lower extremities, n (%)	268 (39.2)	0.62 (0.50-0.76)	<0.001	0.76 (0.35-1.62)	0.468
Upper & lower vs. lower extremities, n (%)	222 (44.6)	0.72 (0.57-0.92)	0.009	1.01 (0.46-2.26)	0.972
Symmetric vs. asymmetric, n (%)	415 (69.6)	0.93 (0.79-1.10)	0.396	0.89 (0.51-1.55)	0.687
SJC, mean (SD)	9.5 (7.4)	0.99 (0.98-1.00)	0.010	0.99 (0.96-1.02)	0.379
CRP (mg/L), mean (SD)	30.4 (34.7)	1.01 (1.00-1.01)*	<0.001	1.005 (1.997-1.013)	0.210
ESR (mm/1hr), mean (SD)	39.7 (27.4)	1.01 (1.01-1.01)*	<0.001	1.005 (0.995-1.015)	0.314
IgM-RF positive, n (%)	378 (58.0)	1.76 (1.50-2.02)	<0.001	6.66 (3.69-12.02)	<0.001
Anti-CCP2 positive, n (%)	217 (32.1)	2.31 (2.00-2.67)	<0.001	11.46 (5.85-22.46)	<0.001
Anti-MCV positive, n(%)	373 (54.6)	1.97 (1.68-2.30)	<0.001	6.13 (3.48-10.79)	<0.001
HLA-SE positive, n (%)	393 (63.8)	1.31 (1.12-1.52)	0.001	2.25 (1.35-3.74)	0.002
CD40 (rs4810485) non-G carrier, n (%)	22 (4.4)	1.02 (0.67-1.58)	0.915	0.78 (0.17-3.54)	0.751

*Outcome of analysis without interaction with time, evaluating whether a factor has an effect on the progression rate that is stable over time. Age, BMI, ESR, SJC, CRP, symptom duration at first visit and morning stiffness were analyzed as continuous variables; this means that the presented OR indicates the odds per unit. For instance, a beta of 1.01 for CRP indicates a 1.01 times higher progression of SHS-score per mg/L CRP. From all 676 patients data on the rate of joint destruction was available, the remission/persistency state was reliably determined in 491 patients

individual factors in the multivariate analysis to the total effect. The proportional effect size of these variables is depicted in Figure 1.

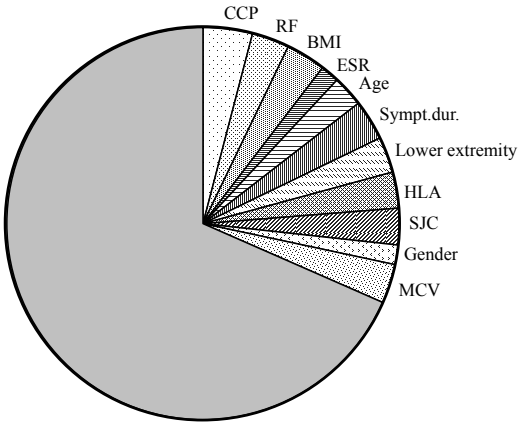

Figure 1. Contribution of baseline variables to the explained variance of Sharp-van der Heijde score over five years. Presented is the explained variance at 5 years of baseline variables that were associated with the progression of joint destruction. CCP: anti-cyclic citrullinated peptide antibody; RF: rheumatoid factor; BMI: Body Mass Index; ESR: erythrocyte sedimentation rate, sympt.dur.: symptom duration at first visit; lower extremity: initial complaints at lower extremity versus upper extremities; HLA: HLA-DRB1 shared epitope alleles; SJC: swollen joint count; MCV: anti-modified citrullinated vimentin antibodies. All continuous variables were categorized in two groups in order to derive this figure: BMI was grouped in lower or higher than 25; Symptom duration at first visit (sympt.dur.) was grouped in lower or higher than 12 weeks; SJC was grouped lower and above 6 swollen joints; Age under and above the median of 57 years; ESR normal or elevated according to reference value

DISCUSSION

Cumulating evidence supports the relevance of initiating DMARD therapy as early as possible. Individualized treatment decision making is hampered by the variability of the outcome of early arthritis. In case of early undifferentiated arthritis, the question is when DMARD therapy should be initiated. In early RA it would be beneficial to recognize the patients who will have a severe disease course, since in these patients the benefits of early combination therapy with potent targeted therapies will up weight the associated costs and risks on side effects. In this themed issue risk factors for the outcome of UA and RA patients are explored based on data of the Leiden EAC.

With regards to early UA it was observed that predictive factors for the fulfillment of the 1987 ACR RA criteria and for having a persistent arthritis were largely similar. A predictive tool for RA development was derived before using a combination of identified risk factors.[10] This prediction rule is now well-validated.[14-16] Since the present study did not intend to re-derive or improve this predictive tool, no multivariate regression analyses were performed in UA patients. Some studies tried to improve this prediction rule and assessed the additive value of baseline erosiveness and genetic markers.[8,17] Unfortunately, these attempts did not result in an increased prognostic performance of this model. Further improvements of the model may be expected to come from ultrasound and MR imaging studies. Although at present not much data on US and MRI in unselected populations of UA patients are available, initial results are promising.[18]

Fulfilling the 1987 ACR criteria as outcome of UA has the disadvantage that it may introduce some circle reasoning; in contrast the difficulty with the outcome measure disease persistency is that classification depends on the duration of follow-up. In UA patients included in this study remission was achieved after a median period of 17 months, whereas in the RA patients the median disease duration till remission was 40 months. A too early comparison of disease outcomes may result in misclassification of potential remission patients into the persistent disease category. In order to diminish the risk on misclassification, in this study we chose to classify patients with ≥5 years of arthritis as being persistent. This follow-up duration is arbitrary and results may have been slightly different in case a shorter or longer follow-up period was chosen.

The most potent predictors for having a persistent course of arthritis in UA patients and a persistent course of RA were the presence of auto-antibodies. Inflammatory markers (the number of swollen joints, ESR and CRP) were associated with the development of RA and a persistent form of arthritis in UA patients as well as the severity of joint destruction in RA patients, which is in line with findings in older studies. However, no significant association between these inflammatory markers and disease persistency was found in RA patients.[19,20] This may be due to the fact that the number of patients with sustained DMARD-free remission in RA was low, thereby reducing the power to identify significant associations with this outcome measure.

It is interesting to note that morning stiffness is strongly associated with the development of RA but not with disease persistency or the severity of joint destruction. Several explanations may account for this feature. One of them is that morning stiffness is mainly related to RA according the 1987 criteria because of circle reasoning. Morning stiffness is not part of the 2010 EULAR/ACR criteria for RA and it would be an interesting subject for further studies to see whether the association between morning stiffness and the risk on RA is still present when the new definition of RA is used.

Other intriguing findings concern the observations on BMI. Obese RA patients are found to have less severe joint destruction. This observation was not only observed in the present study but also in other populations.[21-23] The present study revealed that BMI was not associated with progression from UA to RA, but it was associated with having a persistent arthritis or persistent RA. Thus this indicates that obese patients have more often a persistent disease than non-obese arthritis patients. This observation is highly fascinating and may point to the notion that the role of fat tissue in rheumatoid arthritis is incompletely clear. Fat tissue secretes pro-inflammatory as well as anti-inflammatory adipocytokines.[24] It is clear that some of the mechanisms of joint destruction like osteoclast activation are different than inflammatory pathways and as such it is tempting to speculate that diverse adipocytokines may have different preferential effects on arthritis persistency and on and joint destruction.

The associations between disease outcomes and involvement of the joints of the lower or upper extremities were different for patients with UA and RA. Whereas within UA presence of arthritis on lower extremities was associated with a lower OR on RA, within RA patients it was associated with a higher rate of joint destruction. This finding is in line with previous findings demon-

strating that patients presenting with knee arthritis had a more severe rate of joint destruction compared to patients without knee arthritis, when measured using destruction of small feet and hands joint.[25]

Emerging evidence indicates that anti-CCP positive and anti-CCP negative RA are subsets of RA with differences in the underlying pathologic mechanisms.[26,27] The present study addressed all UA patients and RA patients; stratified analyses on anti-CCP positive and anti-CCP negative patients were not performed. This may be an explanation why *CD40*, a genetic risk factor joint destruction in anti-CCP positive RA is not associated with the rate of joint destruction in the whole RA population.[13]

The baseline characteristics associated with the severity of joint destruction in RA were mainly auto-antibodies and other patient characteristics and to a lower extend factors expressing the level of inflammation. Although the present study did not evaluate the contribution of inflammation over time on the final level of joint destruction, such analyses have been performed before. Some of these studies also suggested that the largest part of joint destruction is not directly related to cumulative inflammatory markers.[28]

The data presented are limited to data of the Leiden Early Arthritis Clinic cohort. However, many of the associating risk factors for UA and RA are observed in individual studies originating from different early arthritis cohorts as well.[29-34]

The proportion of the explained variance in progression of joint destruction by the identified risk factors was 32%. Although no clear guidelines are available what the level of variance explained is required in order to derive a prediction model with an adequate discriminative performance, previous investigations and experience[10,35] are highly suggestive that the explained variance is insufficient to proceed with a derivation of a prediction rule for the rate of joint destruction in RA. This notion is exemplified by recent attempts to derive prediction models; with the current prediction rules about 50% of the RA patients could not be adequately classified.[35-37]

In conclusion, although the processes determining the persistency and severity of arthritis are incompletely understood, the identification of risk factors may help in individualization of therapy in patients with recent-onset UA. In RA, in contrast, the currently known risk factors for a progressive destructive disease course explain only part of the individual differences in level of joint destruction and more risk factors need to be identified in order to achieve at individualized treatment decision making.

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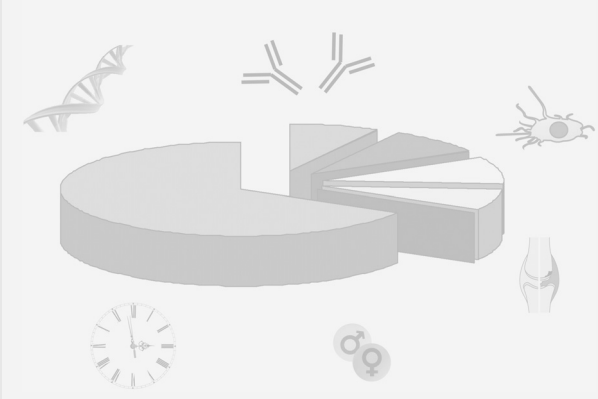
CHAPTER 15



Summary and Discussion

SUMMARY AND DISCUSSION

RA is a chronic and progressive autoimmune disease affecting approximately 1% of the population worldwide, and has a large risk for causing disability of patients and consequently high costs in health care if left un- or improperly treated. To prevent this, patients with RA need to be identified as early as possible and treated adequately to prevent worse outcome. Early recognition, together with prediction of the disease outcome at the individual patient level would allow to achieve personalized medicine. The main scope of this thesis was to identify and evaluate the quality of risk factors for their usefulness in predicting disease course and outcome of RA. To this end, characteristics and disease outcome of RA patients included in the Leiden EAC were studied.

To treat patients with RA adequately and swiftly, the first requirement is a tool to correctly identify patients with early RA. The benefit of early detection and treatment has been recognized many years ago. Although the 1987 classification criteria for RA incorporated a minimally required symptom duration of 6 weeks, the majority of the items of these criteria relate to long standing RA.[1] Although the 1987 criteria have been used for many years and were considered as a huge improvement over the criteria for RA that were formulated in the 1950s,[2] still, the 1987 criteria perform rather poorly in defining RA in a early disease phase.[3,4] The advancing knowledge of the disease course of RA and the need to perform trials in early RA have led to the development of a new set of criteria. These 2010 ACR/EULAR criteria, which now include acute phase reactants and an updated item serology, were devised with the intention to classify patients in an earlier stage of the disease.[5]

Although in the process of deriving the new criteria multiple datasets have been used,[6] their performance in various individual datasets will be the focus of attention in the coming years. Our study (**chapter 13**) indicates that the 2010 criteria, in comparison to the 1987 criteria, classify more patients as having RA. Most importantly, these patients are indeed classified in an earlier stage of the disease. The observation however that an increased number of patients is classified with RA, may indicate a certain degree of false positive misclassification, an expectation that we seem to confirm with our findings. We observed that, depending on the population, 9 or 18% fulfilled other diagnoses during their first year of the disease. Psoriatic arthritis was the most frequent cause of "misclassification". Although the user manual of the 2010 criteria clearly states that these new criteria should only be applied to a set of patients that cannot be classified with another rheumatologic diagnosis, it would be most interesting to be able to use these criteria for every new arthritis patient that visits a rheumatologist. Another issue is that the presence of erosions is *'prima facie'* evidence for the classification of RA within the new criteria, meaning that the other criteria do not have to be fulfilled.[5] Although not a focus of attention in this thesis, the exact definition of an erosion typically for RA should be defined. This has to some extent already been the subject of discussion.[7] A third issue that has not been settled yet, is how an increased RF

level can be defined. As shown in **chapter 4**, the variance in IgM-RF level obtained with different methods is considerable. When evaluating three different outcome measures (MTX initiation during the first year, initiation of any DMARD during the first year and arthritis persistency over 5 years), the new criteria showed a better sensitivity but a lower specificity for the outcome of RA compared to the 1987 criteria. However, since this is the first study published on this topic, replication in other cohorts is needed. Nonetheless, the 2010 criteria could well be regarded as a first step towards earlier recognition of patients at risk and the development of a more accurate set of criteria.

The importance of an earlier detection of RA has been clearly shown by various studies that observed beneficial effects of treating patients as soon as possible. The increase in evidence during the last decade for the existence of a so-called "window of opportunity' of 3 months in which RA patients are most sensitive to treatment,[8-10] is a good example of the growing awareness of the importance to treat early. The effect of treatment within this 3 month period on the effectiveness of response to therapy has been established with significant associations in terms of better outcomes of RA.[11,12] In particular, Finckh et al[13] indicate that early treatment (<3 months) would not only limit the amount of joint damage that can accumulate prior to treatment but in addition it can also slow down the rate of progression afterwards. Although the period of 12 weeks may be somewhat arbitrary, evaluating the effect of early assessment (<12 weeks) in the present study yielded significant support for the importance to treat early (<12 weeks) in terms of long-term outcome of the rate of joint destruction over 6 years and the achievement of sustained DMARD-free remission (**chapter 9**), thereby strengthening prior investigations. Studying the effect of delay on the rate of joint destruction in ACPA subsets showed that the observed association remains present in the ACPA-negative group of patients. Notably, although we did not unequivocally establish a similar result in the ACPA-positive patients, a similar tendency however was observed (**chapter 9**). Although it needs to be considered that this is an observational study and not a randomized trial so it may be that these patients had an innate difference in outcome, we have not been able to identify such differences. Extension of the radiographic data in **chapter 10**, revealed that the effect of delayed assessment on the rate of joint destruction was statistically significant as well in ACPA-positive RA. This indicates that the suggested the "window of opportunity" might apply to both ACPA-negative and -positive RA, but that we lack sufficient statistical power (**chapter 9**). We also observed that among all early arthritis patients ACPA-positive RA patients had the longest delay, and at least 77% of them were assessed after 12 weeks of symptoms. We evaluated whether the "window of opportunity" was explained by different characteristics of the ACPA response (**chapter 10**) and observed that patients that were assessed within or after 12 weeks of symptoms had comparable numbers of isotypes or recognized peptides. The observed lack of an association between the broadness of the ACPA-response and the groups with <12 weeks and ≥12 weeks of delay, might indicate that maturation of the autoantibody response occurs even earlier. This notion would be in line with the observation that autoantibodies, among which ACPA, are already present in the serum of future RA patients in the preclinical phase

several years before the onset of symptoms.[14] The observations that the autoantibody response appears to be initiated before symptom onset, and that levels of acute phase reactants and markers reflecting alteration of bone metabolism are simultaneously elevated as well,[15-17] could lead to the hypothesis that the "window of opportunity" does not lie in the first 12 weeks after initiation of the first symptoms, but is actually located before the clinical onset of disease.

Having established a solid foundation for the need to treat early it is important to raise the question where the observed delay comes from. Since the EAC is organized such that referred patients can visit the rheumatologist quickly (~1-2 weeks), the duration of the delay time, defined as the time between the onset of symptoms and the first visit at a rheumatologist, can in this case be roughly divided into two parts: it could lie either at the patients' end, by reluctance to seek medical care, or at the end of their general practitioner (GP), by referring a patient in a later stage than would be preferred. Although this subject has been studied before and to some extent is subject to geographical variation and depending on the organisation of health care in a particular nation, our study shows that the main contributor to delay in assessment in the Netherlands is the GP delay (chapter 9). An important element in this study was to identify a profile that characterises the patients that have the longest delay. Notably, strikingly similar to the classical image of RA,[18] among all early arthritis patients the current observations were that older age, female sex, gradual symptom onset, involvement of the small joints, lower levels of C-reactive protein, and the presence of autoantibodies were associated with longer total delay. Thus although confirming the general idea about RA, this indicates the need for active and increased awareness to decrease delay time in the future.

The second step in achieving personalized medicine, after early recognition of RA, is to obtain the ability to predict the long-term outcome of RA. To identify risk factors, to this end, two main outcomes used to identify new risk factors for the severity of RA were studied: the rate of joint destruction and the achievement of DMARD-free remission. In this thesis we analysed longitudinal data using a powerful statistical approach (**chapter 2**) that takes maximal advantage of the presence of serial radiographs observed in studies. The ultimate purpose of identifying risk factors is to put together a risk profile for the individual patient, that can lead to the composition of an adequate prediction rule.[19,20]

Although some studies on genetic risk factors for the severity of RA have been performed, fairly little is known about this subject. Most importantly, most observations done thus far, are single studies which have not been replicated. Years of experience however indicate that the replication is in fact needed to prevent false positive findings.[21] In addition, false negative findings should be avoided. Since the effect sizes observed in genetics association studies are in general small, effects may be lost in case of too much noise.

In **chapter 7**, we show that the minor alleles in two loci, rs675520 and rs9376293, located on chromosome 6q23 in a region close to the gene encoding for *TNFAIP3*, associate with a higher

rate of joint destruction within ACPA-positive RA. Since this was the first study to show an association between SNPs located in the *TNFAIP3* region, replication of our findings for the effect on the severity of RA however is needed to confirm the validity of our findings. Nonetheless, when looking at the role of *TNFAIP3* region in RA susceptibility, it can be suggested that the region has similar influences not only on the risk for development but also for a worse outcome of RA, and that the effect in both cases is confined to the ACPA-positive subset of RA patients. For two previously identified susceptibility risk loci in this locus, rs6920220 and rs10499194,[22,23] no effect on the rate of joint destruction was observed however.

A similar observation was done for *PTPN22*, a genetic region previously identified as a risk factor for susceptibility in ACPA-positive RA. As described in **chapter 9**, no association was found between the polymorphism and the rate of joint destruction. Notably, this observation was done in two independent cohorts and thereby provides further indication that *PTPN22* does not affect the development of damage to the joints of RA patients, but may primarily have a role in predisposing to the emergence of ACPA.[24] Other risk factors that also showed similar discrepancies between the risk for susceptibility and severity are *KIF5A*/*PIP4K2C*, *CDK6, CCL21*, *PRKCQ*, and *MMEL1*/*TNFRSF14* (**chapter 8**). Taken together, these observations might indicate that a risk factor for susceptibility is not necessarily always a risk factor for severity of joint damage in RA as well.

Inconsistent effects for RA susceptibility and severity were also found in **chapter 8**, where we investigated the effect of a SNP in the *CD40* gene (rs4810485) on the rate of joint destruction in ACPA-positive patients. Our results show that, in two independent cohorts, ACPA-positive RA patients homozygous for the minor T allele were characterized by significantly higher rates of joint destruction. However, counterintuitive to what one would expect, associating with a less severe course of RA, the common (G) allele conferred risk to develop RA.[25] As pointed out in this chapter, the disease-associated (common) allele marks a haplotype of *CD40* that contains a polymorphism in the upstream Kozak sequence that results in increased surface expression on B cells.[26] In addition, it has been reported that CD40 expression is increased on synoviocytes in RA, and triggering of CD40 in synovial fibroblasts is associated with the production of proinflammatory cytokines and osteoclastogenesis.[27,28] The likeliness that the biologic pathways underlying susceptibility and severity are distinct, in this case with respect to the triggering of CD40, would provide an explanation for the observed discrepancy and in theory could, at least partially, provide an alternative explanation for the discrepancies that are observed for other polymorphisms.

A special role in the pathogenesis of RA is fulfilled by the presence of autoantibodies. These autoantibodies characterize the derailment of the autoimmune system, intended to protect the human body from allogenic threats, by showing a cross-reaction with autoantigens and a subsequent activation of immune responses. Presence of these autoantibodies has firmly been established as associating factors with increased development and severity of RA.[29]

Various tests (IgM-RF, anti-CCP2, anti-CCP3 and anti-MCV) have been manufactured and all have shown to be useful in the process of detecting autoantibodies present during the process of RA. These tests have all individually been reported to have adequate characteristics in terms of performance in terms of sensitivity and specificity, but a head-to-head comparison has never been performed. Although overall anti-CCP2 tended to have the best performance, we find that, evaluating all these tests for a positive or negative test result, no large differences were observed between either test (**chapter 3**) for both the development of RA as well as the rate of joint destruction and the achievement of DMARD-free remission. These results are not surprising since the proportion of patients with presence of more than 1 autoantibody was over 71%, indicating a large coexistence of these autoantibodies. Presence of more than one autoantibody however was associated with worse outcomes. In addition to the presence of ACPA, IgM-RF did not have a significant additive contribution. This also suggests that the predictive value of ACPA is larger than that of IgM-RF.

In the updated item serology of the new 2010 criteria now also the use of ACPA was included in addition to RF.[5] Notably, in addition to the mere presence, the levels of these autoantibodies were given weight as well in the process of classifying a patient with RA. Although higher levels of autoantibodies have been shown to display a higher specificity and associate with an increased development and a higher severity of RA than autoantibody positivity,[30-32] we show that the presence of ACPA also performs better than raising the used cutoff for RF-positivity (**chapter 4**) in addition to the presence of RF (**chapter 3)**. In **chapter 4**, the presence of ACPA performed better for predicting the development as well as the outcome of RA. Moreover, performing a RF test in ACPA-negative patients did not prove to be valuable, while determining ACPA in RF negative patients did contribute. Therefore, we propose to omit the use of RF from the 2010 criteria.

IgM-RF is frequently observed in other inflammatory diseases[33,34] and is sometimes present in healthy older persons,[35] suggesting that RF can be a consequence of nonspecific immune activation. In contrast to IgM-RF, antibodies to anti-citrullinated proteins are highly specific for RA.[36] It has been suggested that IgM-RF production is a consequence of the rheumatoid inflammation whereas ACPA may have pathophysiological properties. Moreover, it is presumed that the association of RF with the presence of RA is primarily explained by its interaction with ACPAs.[37] However, formal proofs that ACPA are causal for RA are lacking.

It has been hypothesized that two different subsets of RA can be characterized by the presence or absence of ACPA.[38,39] This hypothesis is supported by the observed differences in risk profiles for both genetic factors (references,[25,40,41] **chapter7 and 8**)), environmental factors[42] and their interactions, as well as a different reaction to methotrexate treatment in both subsets.[43] Fully understanding the differences between ACPA-positive and ACPA-negative RA as separate entities, especially the underlying molecular pathophysiology, might elucidate the etiology of RA.

Inflammation of the synovium in a rheumatoid joint is a key process in RA, and the intensity and duration of such an inflammation is largely depending on the interplay between different

cell types of the immune system that are localized either in the joints, like fibroblast-like synoviocytes, and cells that roam the human body and are attracted to sites of inflammation, like dendritic cells, macrophages and B- and T-lymphocytes.[44,45]

In chapter 5, we show that the CXCL13, a cytokine that selectively attracts B cells[46], significantly associates with the amount of joint damage in terms of erosiveness and the total Sharp/van der Heijde score. Higher serum CXCL13 levels corresponded with higher rates of joint destruction. The effect was independent of the inflammatory marker CRP, with which the level of CXCL13 on itself is also correlated. Subsequent treatment with anti-TNFα therapy has been reported to significantly reduce CXCL13 serum levels.[47,48] Evidence for joint localization of CXCL13 has been found, both by the detection of mRNA in inflamed synovial tissue[49] as well as the presence of ectopic lymphoid follicles expressing CXCL13 in the synovium of chronic RA patients.[50] Importantly, formation of these ectopic lymphoid follicles has been implicated in initiating and maintaining the inflammatory response in RA.[51] In addition, they have been suggested to associate with increased disease severity and accelerated breakdown of self-tolerance,[52] have been attributed a role in the priming and antigen presentation, and possibly contribute to initiating and maintaining the production of ACPAs, although this latter has not been unequivocally established.[53] The observation that CXCL13 expression takes part in the same chain of events leading to the formation of ACPA, together with the data establishing ACPA as one of the strongest predictors for joint damage, could explain our observation that CXCL13 only shows an association in ACPA-negative RA and that the association is lost in ACPA-positive disease. Notably, high levels of CXCR5 (the CXCL13 receptor) were also found on human osteoblasts and activation by its ligand CXCL13 induced the release of extracellular matrix degrading enzymes. As such, CXCL13 may play a direct role in the process of bone remodeling as well.[54]

The involvement of *TNFAIP3* and *CD40,* genetic regions associated with the rate of joint destruction in RA (**chapters 7 and 8**) as well as susceptibility to RA, together with recent discoveries of other genetic associations -for RA susceptibility- with several genes relevant to this pathway, *TRAF1* and *REL*, especially in autoantibody-positive RA, might point to a central important role of the CD40/NF-κB signaling pathway.[55] As such, to increase understanding of the pathophysiology underlying RA, identification of the cell types that mainly drive this pathway would be of great interest and would propose new interesting targets for interrupting the disease process in RA.

The observed expression of CD40 on the surface of multiple immune cells, including the B-cells, might implicate that CD40 has a broader role in autoimmune regulation in general.[56] Notably, the risk genotype of *CD40* that associates with RA susceptibility but has a protective effect for RA severity (**chapter 8**), has been observed to cause enhanced expression of CD40 on B-cells in Graves' disease.[26] Interestingly, in RA, interaction of CD40 with its ligand, CD40L (CD154), potentially leads to various immune reactions. These include B-cell proliferation through regulation of CDK6 expression, selective attraction of B-cells by regulating CXCL13, germinal center formation, differentiation of B-cells into plasma cells that secrete large titers of

high-affinity antibodies, immunoglobulin class switching, memory B-cell development,[57-61] and affecting osteoclastogenesis by NF-κB/CD40-mediated bone destruction. The sustained presence of the IgM isoform of anti-CCP during the ACPA response that is observed early in the course of ACPA-positive RA, is indicative for ongoing recruitment of new B cells.[62]

Altogether, these findings are supportive for the notion that especially the recruitment and organization of B-cells in the synovium play a critical role in the persistency of arthritis in ACPA-positive RA. This would support the hypothesis of ACPA-positive RA being a B-cell driven disease that was first postulated more than a decade ago.[63] Indeed, modern therapies with B-lymphocyte-depleting agents have shown to be useful in treating ACPA-positive RA.[64,65]

Inflammation is the hallmark of RA and is regarded as the catalyzer leading to disturbances in bone homeostasis by influencing the balance between bone formation by osteoblasts and bone degradation by osteoclasts. This disbalance generally leads to erosions of the joints. The reciprocal processes lead to the occurrence of repair at these sites. Although the concept of repair is still less well accepted, the results from our effort to characterize the subphenotype of RA patients with repair (**chapter 11),** support the notion that repair does exist. In 7.2% of RA patients we observed radiological repair in one or more joints of the same patient. In addition, our results show that despite the absence of aggressive or biological anti-rheumatic treatment, repair occurs in part of the general RA population. Notably, the most frequent occurrence of repair was in the patients who had the highest degree of radiological damage. As mentioned, one of the explanations could be that, to detect this phenomenon, a relatively high degree of eroded bone lesions has to be present (**chapter 11**). This coincides with the observation that in general, the patients with repair simultaneously showed an overall progression in total erosion and Sharp/van der Heijde scores (reference 66 and **chapter 11**).

The observations done on erosions and repair support the idea that not only the occurrence of erosions but also the repair of joint erosions is based rather on the processes involved in local bone homeostasis than on a systemic reaction.[66] Indeed, in our study, repair occurred only in joints without joint swelling in the two preceding years, a finding similar to that done in the TEMPO trial.[67] These observations imply that inflammation drives bone damage, a mechanistical hypothesis that has generally been accepted.[45] Notably, it is recently also suggested that the presence of cartilage and bone breakdown components can induce inflammatory processes.[68] As such a "vicious circle" may be activated.

Thus, the classic paradigm is that inflammation leads to damage, and indeed in majority of cases inflammation goes hand-in-hand with joint destruction. Progressing insights however indicate that the relation between inflammation and joint damage might not be that straight forward and that inflammation and joint damage might have different causal pathways.[68] Evidence substantiating this notion is provided by the observation that, in reaction to treatment an uncoupling of synovitis and joint damage at the individual joint level was observed.[69]

Thorough evaluation of patients with a disconnection between joint inflammation and destruction may yield insight in the processes involved in the link between inflammation and bone destruction. To this end we selected extreme discordant phenotypes (**chapter 12**). We identified patients with persistent joint inflammation over time but after 5 years no erosions (4%), and patients with a very low inflammatory burden but highly progressive joint damage (11%). The high-inflammatory, non-erosive patients were less often autoantibody positive, showed more often an acute start of the disease, and had more inflamed joints. The low-inflammatory, high-erosive patients had a more chronic onset of complaints and were more autoantibody positive. In case of the latter group of patients, it cannot be ruled that subclinical inflammation is present which causes deterioration in of joint damage.[70] It would be very interesting to study whether genetic (rare) variants are associated with these subphenotypes of RA. Although a small number of patients are available, it has been shown in other diseases that studying these extreme discordant phenotypes may be the basis to valuable new findings.[71,72]

Summarizing the data presented in this thesis, in **chapter 14** we provide an overview of the implications of these data for the progression from UA to RA, the development of persistent disease, and the main scope of this thesis, the prediction of outcome in RA in terms of the rate of joint damage. In this study, the risk factors that were observed to associate with progression to RA and the development of persistent disease in UA patients showed to be largely the same, with a main focus on inflammatory markers and autoantibodies. When analyzing risk factors for the outcome of RA, as measured by the rate of joint destruction, the largest effect sizes were observed for the presence of autoantibodies. Other risk factors were inflammatory markers like SJC, CRP and ESR, BMI and, also described in **chapter 9,** the symptom duration (delay) at the first visit to a rheumatologist. Comparison of the identified risk factors for outcome of UA as well as RA again largely resulted in the same set of risk factors. Some risk factors, like a positive family history for RA, acuteness of disease onset, morning stiffness, BMI and several characteristics of joint swelling however were only risk factors for either one of the outcome measures. This observed discrepancy in risk factors for the outcome of UA and RA however is not surprising. We also observed discrepancies between several genetic factors and RA susceptibility and RA severity in **chapter 8** and **chapter 9**.

We show that when combining the individually associating risk factors (**chapter 14**), the overall explained variance for the severity of joint destruction is 32%. During previous attempts to derive prediction rules for the rate of joint destruction, still ~50% of the RA patients could not be adequately classified.[73-75] Although these studies did not include genetics, one can ask the question whether the use of genetics does live up to its expectation of being "the holy grail". For predicting the development of RA from UA it has been observed, that a prediction model incorporating genetic factors did not show an increased performance compared to a prediction rule based on common clinical and serological risk factors alone.[76] Nonetheless, it has unequivocally

been shown that identification of genetic factors, especially in the light of the related concept of heritability, has a substantial influence on understanding the pathophysiology underlying the development of RA.[77,78] The general notion is that for genetics only the tip of the iceberg has been revealed thus far, indicating the need for identification of more and newer genetic risk factors. Moreover, the genetic risk factors identified might not only ultimately allow us to make enhanced prediction of RA development and outcome, but may also give us the opportunity to predict the response to therapy.[79-83]

Our observations might implicate that including genetic factors in predicting the rate of joint destruction can in fact contribute to an increased explanation for the rate of joint destruction, but that for optimal performance, since these factors are primarily identified in the ACPA-positive subset, it would be desirable for future studies to determine the explained variance in the ACPA-positive and ACPA-negative subgroups separately. Next to the inclusion of genetics, also the evaluation of other markers might increase the explained variance of 32% that we observed in **chapter 14**. For example, including CXCL13 (**chapter 5**) will increase the total variance explained, since at the individual level, this factor could explain ~7% of the rate of joint destruction.

In conclusion, huge advances in the understanding and treatment of RA have been made in the last few decades, resulting in dramatically improved perspectives of RA patients nowadays. Nonetheless, the ultimate goal of personalized medicine however has not yet been reached. Although limited in the complete picture of RA, the results described in this thesis may present one step further in the process of achieving individualized treatment decision making. Especially the identification of genetic and serological factors are useful for this purpose. Future studies, dedicated to the identification of more and newer risk factors might help in completing the picture.

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NEDERLANDSE SAMENVATTING

Reumatoïde artritis

Reumatoïde artritis (RA) is een chronische en progressieve ziekte die bij ongeveer 1% van de wereldbevolking voorkomt en wordt gekenmerkt door symmetrische ontsteking van met name de kleine hand en voet gewrichten. Het is een complexe, multifactoriële ziekte en wordt beschouwd als een auto-immuun ziekte door de aanwezigheid van autoantilichamen, zoals tegen gecitrullineerde eiwitten (ACPA), die reageren met het lichaamseigen weefsel. Karakteristiek voor RA is het sluipend ontstaan van de gewrichtsontsteking die kan leiden tot lokale gewrichtsschade (boterosie). Klinisch uit RA zich meestal in progressieve klachten van ochtendstijfheid en functionele beperkingen met mogelijk zelfs invaliditeit tot gevolg indien RA niet tijdig behandeld wordt. Een vroege herkenning gevolgd door een snelle en adequate behandeling zou derhalve kunnen helpen het ontwikkelen alsmede het ernstig verlopen van RA te beperken en mogelijk zelfs te voorkomen. Daarnaast zou de mogelijkheid het verloop van de ziekte te kunnen voorspellen, kunnen leiden tot het opstellen van een persoonlijk risicoprofiel met een daarop afgestemde behandeling voor de individuele patiënt. In dit proefschrift wordt gekeken naar deze voorspellende factoren met als doel het identificeren van nieuwe factoren die kunnen worden gebruikt voor het voorspellen van het verloop van RA.

Vroege herkenning

Om RA te kunnen classificeren werd tot recentelijk gebruik gemaakt van een set criteria uit 1987, bestaande uit aanwezigheid van ochtendstijfheid (>1 uur), symmetrische artritis in >3 gewrichtsgebieden met in ieder geval >1 gezwollen handgewricht, aanwezigheid van reumanoduli, een positieve reumafactor test (autoantilichaam) en erosieve afwijkingen op een röntgenfoto. Voor de classificatie van artritis als RA moest worden voldaan aan 4/7 criteria en moesten enkele criteria langere tijd (>6 weken) aanwezig zijn. Voortschrijdend inzicht heeft recentelijk geleid tot de ontwikkeling van de '2010 revised ACR/EULAR criteria for RA', een nieuwe set criteria met als oogmerk een vroege classificatie van RA in vergelijking met de oude criteriaset. Een directe vergelijking in hoofdstuk 13 met de criteria uit 1987 laat zien dat volgens de 2010 criteria de ziekte inderdaad in een vroeger stadium als RA kan worden geclassificeerd.

Ondanks een toegenomen bewustwording van de noodzaak tot vroege behandeling door een reumatoloog was over de oorzaken en het effect op de uitkomst van RA die wordt veroorzaakt door een vertraging in het bezoeken van een reumatoloog na het optreden van gewrichtsklachten weinig bekend. Zoals is te zien in hoofdstuk 9 wordt slechts 31% van de patiënten binnen 12 weken door een reumatoloog gezien en is een langere vertraging in het bezoeken van de reumatoloog geassocieerd met een slechtere uitkomst van RA, onafhankelijk van de behandeling die de patiënt uiteindelijk kreeg. De vertraging is op te delen in twee onderdelen, namelijk de tijd van het begin van de klachten tot het bezoek van de patiënt aan de huisarts en de tijd tussen het bezoek aan de huisarts en het bezoek aan de reumatoloog. Het blijkt dat in Nederland de patiënt

redelijk snel hulp zoekt (~2-3 weken) maar dat de grootste vertraging zit bij het doorsturen van de patiënt door de huisarts (~8 weken). Enkele belangrijke factoren die samenhangen met het laat doorsturen zijn een oudere leeftijd, vrouwelijk geslacht, het langzaam ontstaan van gewrichtklachten en aanwezigheid van antilichamen; de laatste twee factoren reflecteren het typische beeld van RA. Dit wijst op een noodzaak voor betere herkenning van de kenmerken van RA door de huisarts met als beoogd doel een snellere doorverwijzing van de patiënt bij een klinische verdenking op RA.

Voorspellende factoren

Met het oog op het opstellen van een risicoprofiel en het kunnen voorspellen van de ziekte zijn met name in het afgelopen decennium meerdere risicofactoren geïdentificeerd voor het ontwikkelen van RA. Naast omgevingsgerelateerde factoren zoals roken en alcoholgebruik kunnen ook genetische factoren en aanwezigheid van stoffen die kunnen worden gemeten in het bloed van patiënten (serologie), in het bijzonder autoantilichamen, samenhangen met een verhoogd risico. Over de invloed van zulke factoren op de uitkomst op lange termijn en met name het optreden van gewrichtsschade is echter tot nu toe echter minder bekend.

Een van de vragen in dit proefschrift is wat de invloed is van genetische variatie in gebieden die recent zijn geïdentificeerd als risicofactoren voor het ontwikkelen van autoantilichaam positieve (ACPA-positieve) RA op de progressie van gewrichtsschade. Onze analyses laten zien dat mutaties in *TNFAIP3* (hoofdstuk 6) en *CD40* (hoofdstuk 7) zijn geassocieerd met een slechtere uitkomst van ACPA-positive RA; de onderzochte genetische variatie in *PTPN22* (hoofdstuk 8) heeft echter geen invloed op de progressiesnelheid van gewrichtsschade. Deze resultaten zijn een indicatie dat risicofactoren voor het ontwikkelen van RA niet perse ook een hoger risico geven op een ernstiger beloop.

De aanwezigheid van autoantilichamen vervult een zeer prominente rol bij de pathogenese van RA. Zo is bekend dat aanwezigheid van reumafactor (RF) en de meer recent ontdekte anti-gecitrullineerde antilichamen (ACPA) een zeer sterke associatie hebben met een verhoogd risico op het ontwikkelen van RA en het ontstaan van gewrichtsschade. In hoofdstuk 3 zijn verschillende autoantilichaam testen, namelijk een RF test en drie ACPA testen (anti-gecitrullineerd cyclisch peptide (anti-CCP2&3) en anti-gecitrullineerd vimentine (anti-MCV)) naast elkaar gezet en hun voorspellende eigenschappen vergeleken. Zowel voor het risico op het krijgen van RA als de mate van het ontwikkelen van gewrichtsschade lijkt de aanwezigheid van ACPA, en dan met name de aanwezigheid van anti-CCP2, het meest voorspellend te zijn. Een vergelijking van ACPA met RF laat bovendien zien dat RF op zichzelf weinig lijkt bij te dragen aanvullend op ACPA, een bevinding die wordt ondersteund door de resultaten uit hoofdstuk 4.

Ontsteking vormt een weerspiegeling van een complex samenspel tussen verschillende ontstekingscellen, zoals B en T cellen. Van de stoffen die zij produceren kunnen naast (auto) antilichamen ook andere serologische factoren zoals cytokines mogelijk worden gebruikt bij het voorspellen van het ziekteverloop van RA. In hoofdstuk 5 identificeren we een cytokine die

selectief B cellen aantrekt, CXCL13, als een nieuwe risicofactor voor RA, waarbij een hogere concentratie CXCL13 samenhangt met het ontwikkelen van meer gewrichtsschade.

Ontsteking en schade

Het optreden van gewrichtsontsteking is een van de karakteristieke kenmerken van RA en het optreden ervan wordt gezien als katalysator voor het verstoren van de balans tussen botaanmaak en botafbraak (bothomeostase), die uiteindelijk kan leiden tot het optreden van gewrichtsschade in de vorm van boterosies. Van oudsher is de algemeen geaccepteerde gedachte dat deze vorm van gewrichtsschade irreversibel is en herstel ('repair') niet kan optreden. De recente gedachtenverandering dat 'repair' wel kan optreden, maar slechts infrequent optreedt wordt ondersteund door data in hoofdstuk 11, waar 'repair' wordt gezien bij 7.2% van de bestudeerde RA patiënten. Een van de observaties in de studie was dat deze patiënten 'repair' vertoonden in individuele gewrichten maar tegelijkertijd in de andere gewrichten ook toename van erosies lieten zien, met als gevolg meer progressie in totale schade in vergelijking tot patiënten zonder 'repair'. Dit en het afwezig zijn van zwelling in de gewrichten in de twee jaar voorafgaand aan het optreden van de 'repair' illustreren het lokale karakter van de balans tussen botaanmaak en afbraak.

Het klassieke dogma is dat gewrichtsontsteking de directe oorzaak is van het optreden van de gewrichtsschade. Ondanks dat ontsteking en schade meestal tegelijk worden gezien zijn er echter steeds meer aanwijzingen dat zij niet onlosmakelijk met elkaar verbonden zijn, zogenaamde 'ontkoppeling', en er verschillende oorzakelijke mechanismen aan ten grondslag liggen. In hoofdstuk 12 hebben we patiënten geïdentificeerd die voldoen aan de criteria voor ontkoppeling, te weten patiënten met continu veel gewrichtsontsteking maar na 5 jaar nauwelijks erosies (4%) en patiënten met zeer weinig ontsteking maar veel gewrichtsschade (11%). In vergelijking met de hoog erosieve patiënten kenmerken de laag erosieve patiënten zich door een meer acuut ontstaan van klachten en een verminderde aanwezigheid van autoantilichamen. Het bestuderen van bijvoorbeeld verschillen in genetische factoren bij deze patiënten zou meer inzicht kunnen geven in de relatie tussen ontsteking en schade.

De stand van zaken

In hoofdstuk 14 wordt een samenvatting gegeven van een groot gedeelte van de resultaten die bij de totstandkoming van dit proefschrift zijn verkregen en de implicaties die zij hebben voor het kunnen voorspellen van zowel het ontwikkelen van RA als de uitkomst op langere termijn. Het blijkt dat de risicofactoren voor beide groepen grotendeels overeenkomen, waarvan de aanwezigheid van autoantilichamen het grootste effect heeft op een slechte uitkomst. Daarnaast dragen ook o.a. ontstekingsgerelateerde factoren, zoals gewrichtszwelling en CRP, maar ook het te laat bezoeken van een reumatoloog, zoals beschreven in hoofdstuk 9, bij aan zowel een verhoogd risico voor het ontwikkelen van RA als een ernstig verloop. Combineren we al deze individuele risicofactoren, dan kan ongeveer 32% van de variantie in progressie van gewrichtsschade worden verklaard.

Conclusie

De enorme vooruitgang in het begrip en de behandeling van RA in de laatste decennia heeft geresulteerd in sterk verbeterde perspectieven voor patiënten met RA. Het uiteindelijke doel van op maat gemaakte, persoonlijke geneeskunde is echter nog niet gerealiseerd. Alhoewel in dit proefschrift maar een klein deel van RA als ziektebeeld is belicht zijn de resultaten die worden beschreven wel een stap in de goede richting voor bereiken van geïndividualiseerde behandeling. Toekomstige studies, gewijd aan de identificatie van meer en nieuwere risicofactoren zouden aan het bereiken van het uiteindelijke doel kunnen bijdragen.

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CURRICULUM VITAE

De auteur werd geboren op 28 juli 1978 te Leerdam. Na het behalen van het gymnasium diploma in 1996 aan het gymnasium Camphusianum te Gorinchem begon hij met de studie biologie aan de Universiteit van Utrecht. In 1997 behaalde hij het propedeutisch examen en in hetzelfde jaar volgde hij een excellent tracé, een extra studie onderwerp voor de tien beste studenten met als titel "Eigenschappen van een bacteriële ziekteverwekker". In 1999 startte hij met de opleiding geneeskunde aan het Universitair Medisch Centrum Utrecht. In 2000 werd ook voor de studie geneeskunde het propedeutisch examen behaald. Van november 2002 tot en met juni 2003 deed hij wetenschappelijk onderzoek bij de afdeling hematologie van het Universitair Medisch Centrum Utrecht onder begeleiding van Dr. A. Martens en Dr. H. Rozemuller. In juli 2003 werd het doctoraal examen geneeskunde behaald en in 2005 het artsexamen met succes afgelegd. Voor het vervolg van de studie biologie verrichtte de auteur van november 2005 tot en met september 2006 wetenschappelijk onderzoek bij de afdeling ontwikkelingsbiologie en vergelijkende endocrinologie van de faculteit biologie van de Universiteit Utrecht onder begeleiding van Prof. dr. D.G. de Rooij en Drs. E.A. Abdel Gaber. In maart 2007 werd het schrijven van een scriptie bij de afdeling radiotherapie van het Universitair Medisch Centrum Utrecht onder begeleiding van Dr. H.B. Kal voltooid, waarna in april 2007 de studie biologie eveneens werd afgerond.

Vanaf mei 2007 tot en met november 2007 was de auteur achtereenvolgens werkzaam als arts-assistent neurologie in het Albert Schweitzer ziekenhuis te Dordrecht en als arts-assistent interne geneeskunde/spoedeisende hulp in het Beatrix Ziekenhuis te Gorinchem.

Vanaf 1 december 2007 was hij als arts-onderzoeker verbonden aan de afdeling reumatologie van het Leids Universitair Medisch Centrum (promotor: Prof. dr. T.W.J. Huizinga). Onder leiding van Mw. Dr. A.H.M. van der Helm-van Mil werkte hij aan het onderzoek beschreven in dit proefschrift.

Sinds 1 december 2010 is de auteur in opleiding tot medisch microbioloog in respectievelijk het Sint Antonius Ziekenhuis te Nieuwegein (opleider: Dr. B.M. de Jongh) en het Universitair Medisch Centrum Utrecht (opleider: Dr. A.J.L. van den Bergh-Weersink).

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