Predictive factors for outcome of rheumatoid arthritis

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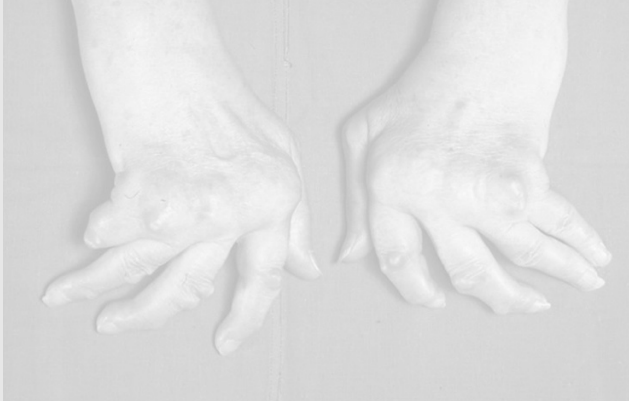

CHAPTER 1

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]

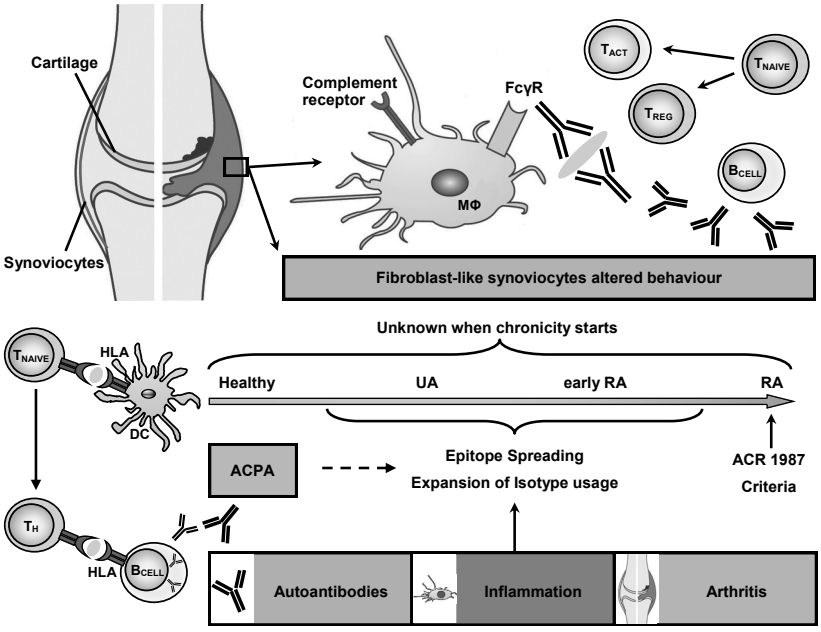

Figure 1. Overview of the complex nature of RA. FcγR: Fc-γ receptor; MΦ: macrophage; T_{ACT}: activated T-cell; T_{REG}: regulatory T-cell; HLA: human leukocyte antigen; T_H: helper T-cell; ACPA: anti-citrullinated protein antibodies; UA: undifferentiated arthritis; RA: rheumatoid arthritis; ACR: American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

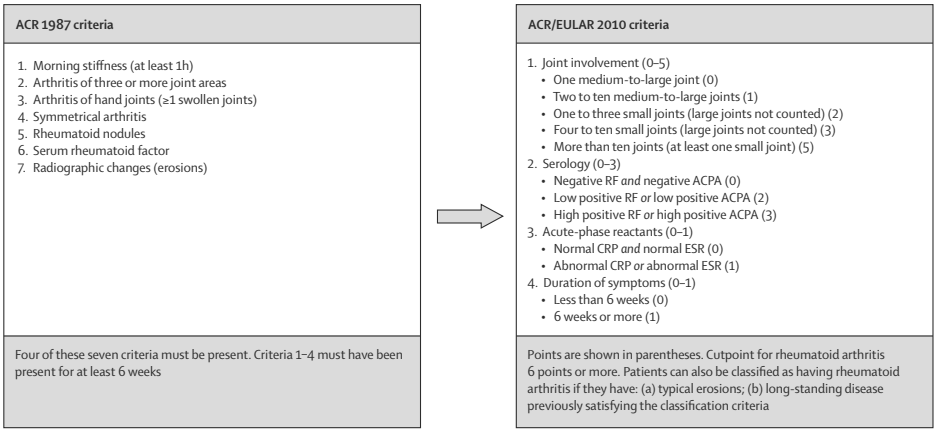

Figure 2. Overview of the 1987 ACR and the 2010 ACR/EULAR criteria. Adapted from Scott et al[4]

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-

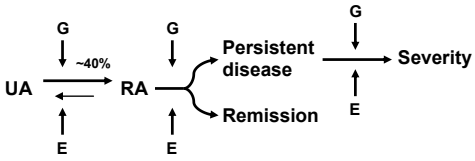

Figure 3. Model of the factors involved in the development and outcome of RA. UA: undifferentiated arthritis; RA: rheumatoid arthritis; G: genes; E: environment

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

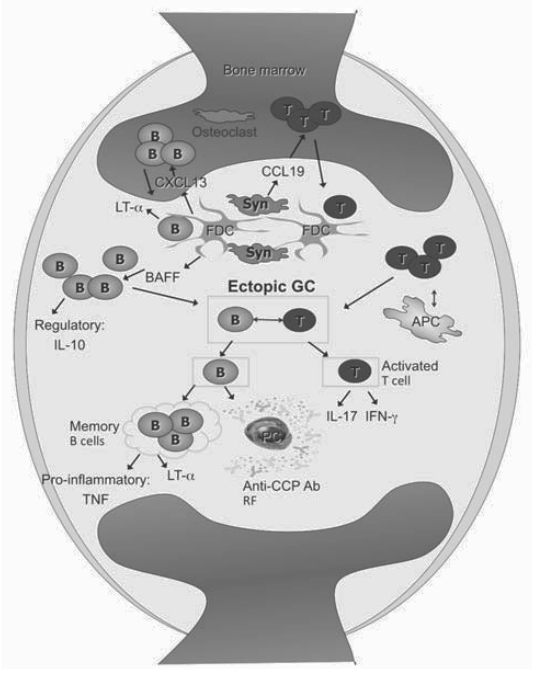

Figure 4. Schematic overview of the inflammatory processes in the rheumatic joint. T: T-lymphocyte; B: B-lymphocyte; Syn: synoviocyte; FDC: follicular dendritic cell; GC: germinal center; APC: antigen presenting cell. Adapted from Marston et al[50]

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]

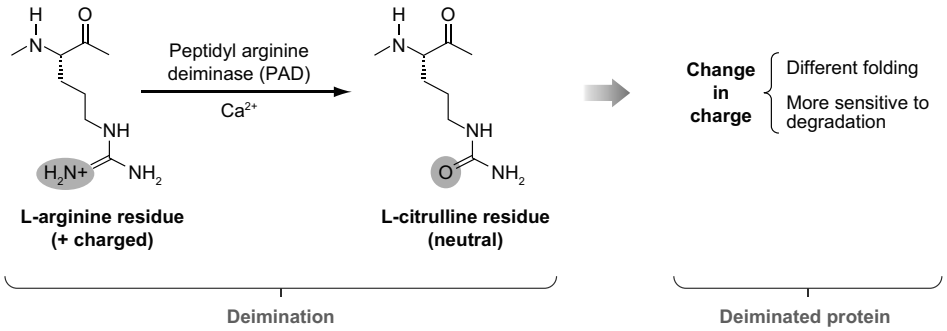

Figure 5. The process of citrullination. Citrullination is an enzymatic conversion that results in the loss of one positive charge for every arginine residue converted to a neutral citrulline, by deimination of peptidylarginine to peptidylcitrulline by the enzyme peptidyl arginine deiminase (PAD). The 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.

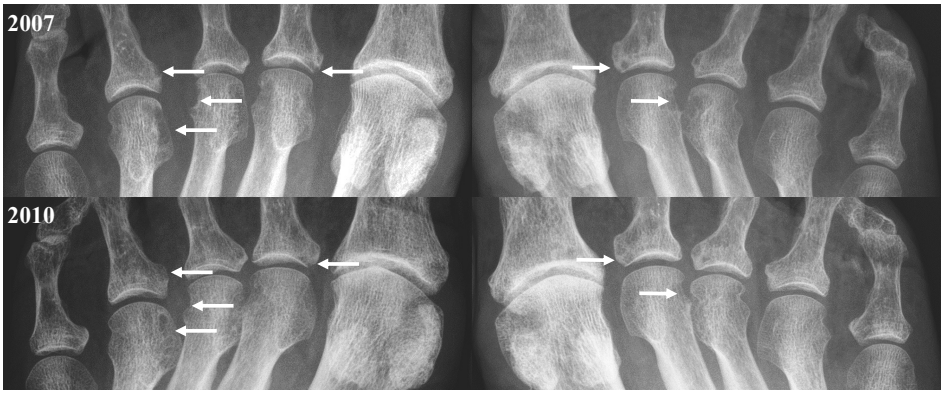

Figure 6. Example of repair of joint erosions. The arrows indicate sites of erosions in the year 2007 in the upper panel. Subsequently, in 2010 shown in the lower panel, these sites have been the subject of new bone formation, so-called repair

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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