CHAPTER 1

Introduction
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RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease primarily affecting small peripheral joints in a symmetrical way, and may have extra-articular manifestations [1]. It is the most common inflammatory arthritis with a prevalence of 0.5 - 1.0% in the adult population worldwide [2]. The disease is approximately three times more frequent in women than in men, and the prevalence increases with age [3]. RA remains to be a major cause of disability, invalidity and reduced quality of life, and is associated with substantial economic costs [4].

CLASSIFICATION OF RA

The 1987 American College of Rheumatology (ACR) classification criteria [5] have been used for many years to identify RA, despite their poor sensitivity and specificity for classification of patients with early RA [6]. They fail to identify individuals with very early inflammatory arthritis who subsequently develop RA [7]. In order to, additionally, facilitate the identification of patients with very early stages of RA a joint initiative of the ACR and the European League Against Rheumatism (EULAR) recently developed the 2010 classification criteria for RA [8]. Those criteria reflect the shift towards earlier diagnosis and treatment of rheumatoid arthritis.

<table>
<thead>
<tr>
<th>ACR 1987 Criteria</th>
<th>ACR/EULAR 2010 Criteria Score</th>
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<tbody>
<tr>
<td>1. Morning stiffness (at least 1h)</td>
<td>1. Joint involvement</td>
<td>0 - 5</td>
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<tr>
<td>2. Arthritis of 3 or more joint areas</td>
<td>• One medium-to-large joint</td>
<td>0</td>
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<tr>
<td>3. Arthritis of hand joints (≥ 1 swollen joints)</td>
<td>• Two to ten medium-to-large joints</td>
<td>1</td>
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<td>4. Symmetrical arthritis</td>
<td>• One to three small joints (large joints not counted)</td>
<td>2</td>
</tr>
<tr>
<td>5. Rheumatoid nodules</td>
<td>• Four to ten small joints (large joints not counted)</td>
<td>3</td>
</tr>
<tr>
<td>6. Serum rheumatoid factor</td>
<td>• More than ten joints (at least one small joint)</td>
<td>5</td>
</tr>
<tr>
<td>7. Radiographic changes (erosions)</td>
<td>2. Serology</td>
<td>0 - 3</td>
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<tr>
<td></td>
<td>• Negative RF and negative ACPA</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>• Low positive RF or low positive ACPA</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>• High positive RF or high positive ACPA</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>3. Acute-phase reactants</td>
<td>0 - 1</td>
</tr>
<tr>
<td></td>
<td>• Normal CRP and Normal ESR</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>• Abnormal CRP or abnormal ESR</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4. Duration of symptoms</td>
<td>0 - 1</td>
</tr>
<tr>
<td></td>
<td>• Less than six weeks</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>• Six weeks or more</td>
<td>1</td>
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</tbody>
</table>

➢ Four of these seven criteria must be present.
➢ Criteria 1-4 must have been present for at least weeks.
➢ Cut-off point for RA is 6 points or more.
➢ Patients can also be classified as having RA if they have: (a) typical erosions; (b) long-standing disease previously satisfying the classification criteria

Table 1: Overview of the 1987 ACR and the 2010 ACR/EULAR criteria
RISK FACTORS AND PATHOGENESIS

RA has a complex etiology; approximately 50% of the risk for developing RA is attributed to genetic factors. Environmental factors (mainly smoking), as well as autoimmunity (mainly Rheumatoid Factor (RF) and anti-citrullinated protein antibodies (ACPA)) contribute to the disease [3].

**Figure 1**: Overview of the pathophysiology and various stages of RA

Genetic risk factors

Genetic factors have a substantial impact on susceptibility to RA. The prevalence of RA in the general population is 0.5% to 1.0%, but it increases to 2% to 4% among siblings of RA probands [9]. The overall genetic contribution to the risk of developing RA has been estimated through studies of monozygotic and dizygotic twin pairs. Cross-sectional twin studies performed on a national scale in Finland and in the United Kingdom found concordance rates for RA of 12.3% and 15.4%, respectively, for monozygotic twins, compared to 3.5% and 3.6% for dizygotic twins [10].
Multiple loci contribute to the genetic risk for RA. The HLA (human leukocyte antigen) locus is the most important of these and accounts for 36% of overall genetic susceptibility to RA [11]. Within the HLA locus, the strongest association is with alleles of HLA-DRB1, which encodes the β-chain of the class II molecule HLA-DR, but recent evidence indicates that other HLA genes also contribute to genetic risk [12]. Outside the HLA locus, the strongest association identified to date is with a polymorphism in the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene, which encodes the protein tyrosine phosphatase Lyp [13]. Advances in genotyping technology have facilitated the application of genome wide association studies (GWAS) to identify disease causal variants. This, coupled with the availability of large case and control collections has enabled the identification of low-to-moderate risk loci. These newer study designs combined with traditional linkage and association studies on single nucleotide polymorphisms (SNPs) level have accelerated the identification of novel RA risk loci (among others STAT4, 6q23 and TRAF1/C5) [11, 14, 15].

**Fceγ-receptors (FceγRs)**

The FceγRs play a crucial role in immunity by linking the IgG antibody mediated responses with cellular effector and regulatory functions [16]. These receptors are encoded by genes clustered on the long arm of chromosome 1 (1q21 - q24) in a complex region showing extensive nucleotide sequence homology that resulted from duplication and recombination events which occurred in this cluster during the evolution [17]. Such genetic complexity renders successful genotyping of different SNPs in that region using classical methods notoriously difficult [18].

Data from mice models revealed that, arthritis-susceptible, DBA/1 mice lacking FcR gamma chain (thus lacking FcγR1 and FcγR3 signaling) were protected from collagen-induced arthritis (CIA) [19]. In contrast, deletion of FcγRII can render arthritis-resistant 129/SvJ and C57BL/6 hybrid mice susceptible to CIA [20]. These observations suggest a crucial role of FcγRs in triggering autoimmune arthritis [19]. Additionally, the balance of activating FcγRs (FcγRI, FcγRIIa, FcγRIlc, FcγRIIia, and FcγRIIib) and inhibitory FcγRs (FcγRIIb) might be important in regulating the chronic immune complex–mediated responses in patients with RA (figure 2) [21].
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Figure 2: Immune reaction is a balance between the inhibitory (FcγRIIb) and activating FcγRs (FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb)

Environmental risk factors

In addition to genetic aspects, environmental risk factors are known to contribute to the etiology of RA. Many epidemiological studies have shown an association between smoking and the development of RA [22, 23]. Smoking was found to interact with the HLA SE (Shared Epitope) alleles in the predisposition to RF-positive RA [24] and ACPA-positive RA [25]. Smoking was associated with the development of citrullinated antigens in broncho-alveolar lavage fluid cells, providing a plausible pathogenic link between smoking and the development of ACPA-positive RA [25]. Other potential environmental risk factors include alcohol intake, coffee intake, vitamin D status, oral contraceptive use, and socioeconomic status although supporting evidence for these other factors is weak [26].

Auto-antibodies

Autoantibody formation is characteristic for RA and that is the reason it is considered an autoimmune disease. The positive predictive value of the presence of certain antibodies aims at another phase of the immune response that may play a role in the pathophysiology of RA. 50–80% of individuals with rheumatoid arthritis have rheumatoid factor, ACPA, or both [27].

Rheumatoid factor

The classical antibody in RA, rheumatoid factor (RF), is an antibody directed to the Fc part of immunoglobulins. The presence of IgM-RF is part of the 1987 ACR criteria for RA.
However, RF can be detected in other autoimmune diseases and healthy individuals. The sensitivity of RF for diagnosing RA ranges from 60 to 70%; and the specificity from 80 to 90% [28].

**Anti-citrullinated peptide antibodies**

Additional, and increasingly important, types of antibodies are those directed against citrullinated peptides (ACPA) [3]. Although most, but not all, ACPA-positive patients are also positive for RF, ACPA seem more specific and sensitive for diagnosis and seem to be better predictors of poor prognostic features such as progressive joint destruction [29]. Evidence from animal models and in-vivo data suggest that ACPA are pathogenic on the basis of induction of arthritis in rodent models and because immunological responses are present in ACPA-positive patients in a citrulline specific manner [30]. Findings of clinical studies show that patients with RA and both RF and ACPA autoantibody-positive disease) differ from individuals with so-called autoantibody-negative disease [3]. For example, histologically, people with ACPA-positive disease have more lymphocytes in synovial tissue, whereas those with ACPA-negative rheumatoid arthritis have more fibrosis and increased thickness of the synovial lining layer [31]. ACPA-positive disease is associated with increased joint damage and low remission rates [32]. ACPAs can be present up to 14 years upon diagnosing rheumatoid arthritis [33, 34].

**Vascular factors**

Recently, the role of the synovial vasculature in the pathogenesis of RA has gained more interest [3]. Data from mouse work lead to the hypothesis that changes in local vascular permeability might provide a route via which a systemic autoimmunity becomes focused on the synovium [35]. Early in RA angiogenesis within the hypertrophic synovial sub-lining occurs and is driven by the increased metabolic demands and hypoxia of the expanding inflammatory tissue [36]. New vessels in the RA pannus are of a characteristic branching morphology [37]. This is thought to result from the unfavorable co-expression by intimal fibroblast-like synoviocytes (FLSs) and vascular endothelial cells (ECs) of the angiogenic factor VEGF (vascular endothelial growth factor), and the angiopoietin-1/2–TIE2 complex [36]. VEGF and angiopoietin-2 together promote the invasive proliferation of ECs, and
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inhibition of this process may be one mechanism of anti-TNFα drug activity [38]. It is hoped that the complex paracrine pathways that promote angiogenesis may yield additional therapeutic targets [39].

UNDIFFERENTIATED ARTHRITIS

Undifferentiated arthritis (UA) is defined as any arthritis of recent onset that cannot be classified according to the existing criteria for specific rheumatic disorders [40]. Patients with early UA form a heterogeneous group exhibiting a variable disease course. Of patients with early UA 40 to 50% remit spontaneously, whereas 30% subsequently fulfill the 1987 ACR criteria therefore classified as RA [41-43]. Since starting treatment with disease-modifying anti-rheumatic drugs (DMARDs) earlier leads to improved outcomes for patients with RA, initiating anti-rheumatic therapy already in the UA phase might result in sustained benefits and potentially remission [44-46]. That implies the need to identify which UA patients will have a chronic destructive disease course and those who will go into spontaneous remission or develop other diagnoses. Several prediction models have been made, yet none is accurate enough that all individual UA patients can be predicted correctly [47, 48].

ASSESSMENT OF RADIOGRAPHIC JOINT DAMAGE

Plain radiographs of hands and feet remain the gold standard of assessing joint damage in RA. Several methods are present for quantifying the amount of joint destruction visible on radiographs. Of these methods, the Sharp/van der Heijde scoring (SHS) is considered as the most sensitive widely used compared to the Larsen and the Ratingen scoring methods [49]. This method focuses on the small joints in the hands, the wrists and the feet. Joint space narrowing (JSN) is scored in 42 joints and is a measure for cartilage degradation. The erosion score (ES) is scored in 44 joints and is a measure of bone degradation Figure 3. The sum of JSN and ES is referred to as the total SHS and can be a maximum score of 448 [50]. In literature, evaluation of radiographic data is frequently performed on a single time point in a cross-sectional way, though, a more precise estimation of individual’s progression rate can be ideally achieved with repeated evaluation of joint damage [51].
Figure 3: Scoring of hands and feet according to the SHS method. Joints and sites scored for joint space narrowing are shown on the left while joints and surfaces of the joints scored for erosions are shown on the right.
AIM AND OUTLINE OF THIS THESIS

The ultimate quest in RA is to fully understand the various risk factors and try to early identify or even predict RA before the criteria for diagnosis are fulfilled. This will permit the early start of DMARDs in identified RA cases or UA predicted to develop RA which was shown to have sustained beneficial effects.

The focus and objective of this thesis is to:

1. Add insight into genetic risk factors of RA in the context of antibody heterogeneity and interaction with environmental, racial and gender factors.
2. Study the diagnostic and prognostic value of the various autoantibodies in RA.
3. Define erosions and their value in prognosis of UA patients, prediction and early diagnosis of RA patients.

Consequently, the thesis is divided into three parts; each addressing one of the key objectives.

Part I of the thesis is devoted to thoroughly study various genetic risk factors of RA. The previously reported genetic risk factors have been explicitly shown to predispose for ACPA-positive RA but not for ACPA-negative RA emphasizing the need to systemically study genetic risk factors in ACPA-positive and ACPA-negative RA separately. The interaction of environmental factors with genetic risk factors should be taken into consideration. Additionally, we will try to approach the technical difficulties and contradicting results from genetic studies encompassing the FcγRs genes.

Even though PTPN22 R620W gene polymorphism has been consistently shown to be associated with RA in Caucasians, it is not found and thus is not disease associated in Asian populations [52]. In chapter 2, we investigated whether RA risk associated with PTPN22 genetic variants in Europeans was solely conveyed through the R620W allele, that is not polymorphic in Asians, or other SNPs and haplotypes play a role in that risk. Other genetic variants were shown to carry risk for RA in Asians but not in Europeans as FCRL3 and PADI4. We were interested to investigate those in Dutch Caucasians and explore whether stratifying for antibody status has an effect on the risk for RA. In chapter 3, we tested the
association of FCRL3 polymorphism with RA susceptibility and severity in Dutch Caucasian patients with RA. Additionally, we performed a meta-analysis to reveal the contribution of this gene to RA susceptibility. In Chapter 4, we tried to elucidate the differential role of PADI4 polymorphism in RA risk between Asian and European populations. Moreover, we explored the possible gene-environmental interactions between the PADI4 polymorphism, sex and smoking status.

The FcγRs genes region has been shown to exhibit copy number variation (CNV) in several large-scale whole genome and focused studies [53-56]. The presence of common CNVs can cause false SNP genotyping results Figure 4. The subsequent skewing of genotypes may lead them to fail the Hardy-Weinberg equation (HWE) (therefore excluding their results) and/or may blur the association of the studied SNPs with disease susceptibility. It may also limit the ability of the genome-wide SNP association studies to detect disease-associated SNPs in regions with CNV [57]. Such genetic complexity renders successful genotyping of different SNPs in that region using classical methods notoriously difficult. Therefore, an alternative approach is required to tackle the genetic variations in this region and its association with RA risk and/or severity.

**Figure 4:** Possible effect of copy number variation (CNV) on single nucleotide polymorphism (SNP) genotyping. This figure illustrates how the presence of CNV can cause false SNP genotyping results. A high copy number falsely enriches the heterozygotes, while the presence of low copy number falsely enriches the homozygotes. In this figure the use of F and V characters was only for illustration purpose but this kind of erroneous genotyping result can occur in every genetic region that harbours both SNPs and CNV.
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In chapter 5, we explored whether FcγRIIIA 158V/F SNP associates differently with ACPA-positive and ACPA-negative RA. The CNV of FcγR genes was studies using Multiplex Ligation Dependent Probe Amplification (MLPA) and we tested whether the FcγRIIIA gene CNV itself confers risk for RA and if the CNV affects the association of the FcγRIIIA 158V/F SNP with RA. Similarly in chapter 6, the genetic variations of FcγRIIIB gene were studied using MLPA in RA patients.

Part II focuses on the autoantibodies formed in the course of RA; the best known of those is the rheumatoid factor. More recently, several tests have been developed that detect ACPA. These tests include the commercially available anti-CCP2 and anti-CCP3 assays as well as the anti-mutated citrullinated vimentin (anti-MCV) test. The first two assays use citrullinated peptide(s) for the detection of ACPA, whereas the later assay is based on an entire protein, citrullinated vimentin [58, 59]. The diagnostic performance of the anti-CCP2, anti-CCP3 and anti-MCV tests in differentiating RA from other forms of arthritis in a clinical setting of early arthritis is investigated in chapter 7.

Finally, Part III of the thesis focuses on bone erosions and its significance in the disease course of RA and UA. The presence of early bone erosions in RA patients is predictive of severe destructive disease course, while its prognostic value in UA is unknown. Additionally, the definition of erosive disease is unclear and different studies use different descriptions and cut-off values. Even though the presence of erosions is part of the 1987 ACR classification criteria for RA [5], it is not specified how many erosions are required and even more, mentions erosions in the hands and not in the feet. Chapter 8 of this thesis aims to: study the predictive value of erosive joints in hands and feet for development of RA in UA-patients; define the optimal number of erosive joints to predict RA; define whether the predictive ability is different between erosive joints in hands and feet; determine whether information on erosive joints increases the discriminative ability of a recently developed prediction rule for RA-development [48, 60]; and investigate whether the results are different when disease persistency is studied instead of the development of RA according to the 1987 ACR criteria.
REFERENCES
31. van Oosterhout, M., et al., Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive rheumatoid arthritis and anti-cyclic
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