General Introduction: the Development of Rheumatoid Arthritis and the Role of Autoantibodies
INTRODUCTION

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease that is characterized by a chronic inflammation of multiple joints. This disease can affect all synovial joints, but is mostly located in the small joints of hand and feet. Patients suffer from joint pain, swelling and stiffness after inactivity. In some cases the disease also manifests itself systematically with low-grade fever, anorexia, fatigue, weight loss, malaise, anaemia, nodules, osteoporosis, lymphadenopathy and many other symptoms. Onset can be acute, but more often is insidious with a waxing and waning character of the complaints. This waxing and waning character makes the disease hard to diagnose, especially in the early phase.

What causes the inflammation of the joints is unknown, but the inflammatory process itself has been studied extensively. Normally, the synovial membrane, which is the inner layer of the joint capsule facing the intraarticular joint space, consists of a thin layer of macrophage–like synoviocytes and fibroblast–like synoviocytes (FLS). The initial inflammatory response in RA is characterized by an inflammation of this synovial membrane, a so–called synovitis. T–cells (mainly CD4+, TH1 biased), B–cells, plasma cells and dendritic cells (DCs) are recruited to the synovium and the thin lining becomes hyperplastic and highly vascularized. The TH1 cells become activated, perhaps through Major Histocompatibility Complex type II (MHC–II) mediated antigen presentation by DCs, and secrete various cytokines, such as interferon–β (INF–β) and interleukins (ILs). These cytokines in turn activate monocytes, macrophages and FLS. Subsequently, these cells start to produce more, predominantly pro–inflammatory cytokines, of which tumour necrosis factor–α (TNF–α) and IL–1 are thought to be the most important in perpetuating inflammation. Responding cells are driven towards a pro–inflammatory phenotype. Macrophages differentiate into osteoclasts that resorb and destroy bone. Chondrocytes and FLS secrete matrix metalloproteinases (MMPs), which cause cartilage degradation. B cells turn into plasma cells that secrete auto–antibodies, such as rheumatoid factor (RF) and anti citrullinated protein antibodies (ACPA). These can form complement fixating immune complexes that activate neutrophils and macrophages, which further contribute to bone destruction. The inflammation of the joint can cause pain and swelling. The bone destruction causes deformity of the joints which can lead to disability. To halt these processes, treatment is essential. In recent years, it has been acknowledged that also the timing of treatment is important. It appears that a ‘window of opportunity’ exists.
early after diagnosis in which there is a greater than usual response to therapy with better long term outcome.\textsuperscript{13-18} Therefore, timing of the diagnosis is also important. However, diagnosis of RA in a very early stage can be difficult. Studying the development of the disease may provide insight in the first stages of the disease and thereby help in early diagnosis.

**Risk factors for rheumatoid arthritis**

As mentioned above, the trigger that starts the inflammatory process in RA is unknown. It is neither known how it appears, nor when it appears. Certain risk factors, such as genetic, hormonal, infectious and environmental factors, are recognized that probably contribute to a susceptibility background against which RA can develop.

Genetic variation is thought to explain 50–60% of the disease liability.\textsuperscript{19} The major genetic risk factor is the shared epitope (SE). This sequence is shared by a few \textit{HLA-DRB1} alleles and encodes an amino acid sequence bordering the peptide binding groove of the human leucocyte antigen (HLA)–DRB1 molecules.\textsuperscript{20,21} HLA–DRB1 molecules harboring this SE supposedly have higher affinity for citrullinated peptides,\textsuperscript{22} antigens implicated in the pathogenesis of RA. Other genetic risk factors include PTPN22, STAT4 and TRAF1–C5.\textsuperscript{23-25}

The influence of hormonal factors seems reflected by a two–fold higher incidence among females in comparison to males and the influence of pregnancy on the disease. However, literature concerning hormonal influences is often contradictory and the exact role of hormones in the development of RA remains unclear.\textsuperscript{26-31}

Infectious agents are considered as candidates to trigger autoimmunity with cross–reactivity. The most suspected candidate is \textit{Porphyromonas (P.) gingivalis}, a bacterium that causes periodontitis, which is associated with RA.\textsuperscript{32} This expresses its own peptidylarginine deiminases (PAD),\textsuperscript{33} the enzyme that citrullinates proteins and peptides. \textit{P gingivalis} expresses bacterial enolase, which has 51% homology with human α–enolase.\textsuperscript{34} Both proteins can be citrullinated by \textit{P gingivalis} PAD and become neoantigens in individuals susceptible to RA.\textsuperscript{35}

The strongest association of RA with environmental factors is with smoking. This association is even stronger is SE positive individuals.\textsuperscript{36} It is hypothesized that smoking causes lung tissue changes such as citrullination, thereby forming neoantigens to which antibodies can be formed, again causing autoimmunity via cross–reactivity.
Presymptomatic phase: autoantibodies

In susceptible individuals, a deregulation of the immune system can occur. This deregulation can present itself with the formation of autoantibodies, such as RF and ACPA. These antibodies can be detected before the disease becomes symptomatic.\cite{37-39} As such, a presymptomatic phase in which deregulation of the immune system is already present, can be recognized. In this phase also other evidence of an activated immune system can be found, such as a slight increase in the acute phase response and an increase in certain chemokines,\cite{40,41} but the major anomaly is the presence of autoantibodies.

RF were the first antibodies described in RA, although they were at first not recognized as antibodies, hence the name factor.\cite{42,43} RF are antibodies directed against the Fc portion of IgG and thereby can form large immune complexes and might thus contribute to the pathogenesis of RA. They can be found in around 75 percent of the patients with RA, but are not very specific for RA since they can also frequently be observed in other inflammatory diseases.\cite{44,45}

ACPA are a group of antibodies directed against citrullinated proteins, first described in 1964 by Nienhuis and Mandema as the antiperinuclear factor.\cite{46} Citrullination is a calcium–dependent post–translational modification that comprises the substitution of peptidylarginine to peptidylcitrulline by PAD. PAD is expressed in synovial tissue,\cite{47} but normally citrullinated proteins are not found in the joints. Under inflammatory conditions however, calcium concentration in dying cells rises and citrullination can occur. The citrullinated proteins may then leak from dying cells, spilling in the extracellular environment. Indeed citrullinated proteins are present in inflamed joints,\cite{48} but can also be found in other tissues, such as the lungs and the gingiva. Examples of citrullinated proteins that are targeted by ACPA are keratin, fillagrin, collagen, fibrinogen, vimentin and α–enolase.\cite{48-55} ACPA can be found in around 70 percent of RA patients and are, unlike RF, highly specific (96%–99%) for RA.\cite{56} This high specificity suggests a pathogenic role for ACPA in RA, which is substantiated by in–vitro and animal data. Immunization with citrullinated type II collagen (CII) in the collagen–induced arthritis (CIA) model leads to a more severe arthritis than immunization with native CII.\cite{57} Administration of monoclonal antibodies to citrullinated fibrinogen enhances CIA, whereas tolerization prior to disease induction can diminish CIA. Furthermore, immunization with CII induces ACPAs, which develop prior to the onset of arthritis.\cite{58}

RF and ACPA are not the only autoantibodies found in RA patients. Other antibody systems exist. An example are anti–carbamylated protein (anti–CarP) antibodies.\cite{59}
These antibodies target carbamylated proteins instead of citrullinated proteins. Carbamylation is a process in which lysines are converted into homocitrullines under the influence of cyanate. Homocitrulline highly resembles citrulline. Cyanate can be formed in low concentrations from urea under physiological conditions or it can originate from the environment, for instance from car fumes. In inflammatory conditions it can be formed from thiocyanate under the influence of myeloperoxidase that is released by for instance activated neutrophils. Whether or not anti–CarP antibodies are directly involved in the pathogenesis of RA is currently unknown.

**Symptomatic phase: arthralgia**

In some individuals that develop autoantibodies such as ACPA, the deregulation of the immune system progresses and they become symptomatic. The cause of this progression is currently unknown, but theoretically some 'second hit' could be necessary.

Often the symptoms start with pain of the joints with sometimes also complaints of morning stiffness. The pain often occurs in multiple joints, sometimes all at once, sometimes successively. Most patients describe intermittent symptoms with periods of frequent ‘attacks’ and quiet periods with few symptoms. Attacks can last as short as a few hours to a few days. In this phase, there are still no visible or tangible abnormalities of the joints and symptoms are therefore referred to as arthralgia. However, because of their symptoms, arthralgia patients do seek help and can come under the attention of a rheumatologist. In arthralgia patients with a positive autoantibody status (seropositive), progression towards arthritis can occur, but symptoms can also diminish or disappear. Therefore, it remains a challenge to predict which patients will develop arthritis.
Table 1. The 1987 revised classification criteria for RA*

<table>
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<tr>
<th>Criterion</th>
<th>Definition</th>
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<tr>
<td>1. Morning stiffness</td>
<td>Morning stiffness in and around the joints, lasting at least one hour before maximal improvement.</td>
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<tr>
<td>2. Arthritis of 3 or more joint areas</td>
<td>At least 3 joint areas (out of 14 possible areas; right or left PIP, MCP, wrist, elbow, knee, ankle, MTP joints) simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) as observed by a physician.</td>
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<tr>
<td>3. Arthritis of hand joints</td>
<td>At least one area swollen (as defined above) in a wrist, MCP, or PIP joint.</td>
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<tr>
<td>4. Symmetric arthritis</td>
<td>Simultaneous involvement of the same joint areas (as defined above) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs, without absolute symmetry is acceptable).</td>
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<tr>
<td>5. Rheumatoid nodules</td>
<td>Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions as observed by a physician.</td>
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<tr>
<td>6. Serum rheumatoid factor</td>
<td>Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in less than 5 percent of normal control subjects.</td>
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<tr>
<td>7. Radiographic changes</td>
<td>Radiographic changes typical of rheumatoid arthritis on posteroanterior hand or wrist radiographs, which must include erosions or unequivocal bony decalcification localised in, or most marked adjacent to, the involved joints (osteoarthritis changes alone do not qualify).</td>
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*For classification purposes, a patient shall be said to have rheumatoid arthritis if he has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks.

Undifferentiated arthritis and rheumatoid arthritis: diagnosis and classification criteria

If a patient develops arthritis, diagnosing RA is not straightforward. Until recently, the 1987 criteria for the classification of RA* were used (Table 1). However, these criteria are developed to classify RA for the purpose of research and rely heavily on the presence of clinical symptoms. Furthermore, the presence of ACPA is not included in these criteria, instead, the presence of RF is. They are therefore not suited for early diagnosis of RA, because in the early phase not all symptoms are clearly present and the presence of ACPA is more often seen than the presence of RF. According to the 1987 criteria, early arthritis would often be classified as undifferentiated arthritis (UA).

Fortunately, various studies have focused on the early phase of RA and different models are presented to help diagnose or predict RA in this phase. Moreover, a joint taskforce of the ACR and EULAR has recently produced new classification criteria for RA (Table 2), developed to classify RA in a very early stage. However, these classification criteria and prediction models still rely on the presence of...
clinically apparent arthritis. A tool to predict the development of RA before arthritis is clinically evident at present does not exist.

Outline of the thesis
The aim of this thesis was to investigate whether and how arthritis development can be predicted in individuals at risk for developing RA, better than by antibody status alone. Therefore, a prospective cohort of seropositive arthralgia patients was followed for the development of arthritis.

To gain more insight in the development of the disease, autoantibody responses were studied in this cohort and also in serum samples of healthy blood donors who later developed RA. Furthermore, to study the characteristics of ACPA, a method was developed to clone monoclonal ACPA out of B cells of RA patients.

In the first part of this thesis the development of the ACPA response and other antibody responses prior to the diagnosis of RA is described. The development of the ACPA response to different antigens is described in chapter 2. The extent of this response is associated with arthritis development, which is reported in chapter 3. In chapter 4, a method is presented to clone monoclonal ACPA derived from an RA patient. This method resulted in a more precise characterization of ACPA. Chapter 5 reports the avidity maturation of ACPA. In chapter 6 and 7 other pre-clinical autoantibodies such as anti carbamylated Protein antibodies (chapter 6), anti-Hinge antibodies (chapter 7), are described.

The second part of this thesis is dedicated to the prediction of RA. In chapter 8, the use of acute phase reactants in the prediction of RA is reported, in chapter 9 the use of the type I INF signature and in chapter 10 the use of lipid levels. In chapter 11 the value of ultrasonography in predicting RA is studied. The finding that alcohol protects against arthritis development is described in chapter 12. In chapter 13 a prediction rule for the development of arthritis is presented.

The results of this thesis are summarized and discussed in chapter 14.
### Table 2. The 2010 ACR–EULAR classification criteria for RA

<table>
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<tr>
<th>Target population:</th>
<th>Score</th>
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<tr>
<td>Patients who have at least 1 joint with definite clinical synovitis (swelling)* with the synovitis not better explained by another disease.</td>
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Classification criteria for RA (score–based algorithm: add score of categories A–D; a score of ≥6/10 is needed for classification of a patient as having definite RA)‡

<table>
<thead>
<tr>
<th>A. Joint involvement§</th>
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<tr>
<td>1 large joint¶</td>
<td>0</td>
</tr>
<tr>
<td>2–10 large joints</td>
<td>1</td>
</tr>
<tr>
<td>1–3 small joints (with or without involvement of large joints)¶</td>
<td>2</td>
</tr>
<tr>
<td>4–10 small joints (with or without involvement of large joints)</td>
<td>3</td>
</tr>
<tr>
<td>&gt;10 joints (at least 1 small joint)**</td>
<td>5</td>
</tr>
</tbody>
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| B. Serology (at least 1 test result is needed for classification)†† |      |
| Negative RF and negative ACPA | 0    |
| Low–positive RF or low–positive ACPA | 2    |
| High–positive RF or high–positive ACPA | 3    |

| C. Acute–phase reactants (at least 1 test result is needed for classification)‡‡ |      |
| Normal CRP and normal ESR | 0    |
| Abnormal CRP or abnormal ESR | 1    |

| D. Duration of symptoms§§ |      |
| <6 weeks | 0 |
| ≥6 weeks | 1 |

* The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

‡ Although patients with a score of <6/10 are not classifiable as having RA, their status can be reassessed and the criteria might be fulfilled cumulatively over time.

§ Joint involvement refers to any swollen or tender joint on examination, which may be confirmed by imaging evidence of synovitis. Distal interphalangeal (IP) joints, first carpometacarpal joints, and first metatarsophalangeal (MTP) joints are excluded from assessment.

¶ "Large joints" refers to shoulders, elbows, hips, knees, and ankles.

# "Small joints" refers to the MCP joints, proximal IP joints, second through fifth MTP joints, thumb IP joints, and wrists.

** In this category, at least 1 of the involved joints must be a small joint; the other joints can include any combination of large and additional small joints, as well as other joints not specifically listed elsewhere (e.g., temporomandibular, acromioclavicular, sternoclavicular, etc.).

†† Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low–positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high–positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low–positive for RF.

ACPA = anti–citrullinated protein antibody.

‡‡ Normal/abnormal is determined by local laboratory standards. CRP = C–reactive protein; ESR = erythrocyte sedimentation rate.

§§ Duration of symptoms refers to patient self-report of the duration of signs or symptoms of synovitis (e.g., pain, swelling, tenderness) of joints that are clinically involved at the time of assessment, regardless of treatment status.
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