

# **Chapter 1**

## **General introduction**



## Rheumatoid arthritis

**R**heumatoid arthritis (RA) is an autoimmune disease, characterised by chronic inflammation of the joints, resulting in pain, stiffness and the loss of function [1]. Women are more affected than men in a ratio 2:1. RA is the most common form of chronic inflammatory polyarthritis, affecting approximately 0.5 to 1% of the Northern European and North American population. Studies in Southern Europe report a lower prevalence of 0.3 to 0.7% [2]. In the Netherlands, the reported prevalence of RA varies between 1.0 and 1.3% [3]. The incidence of RA in the Netherlands is 0.2 to 0.4 per 1000, which is in line with other Northern European and North American countries [3, 4].

The aetiology of RA is unknown, but both genetic and environmental factors are thought to be important in the pathogenesis of RA (reviewed in [5]). The major histocompatibility complex (MHC) genes, which encode the human leukocyte antigens (HLA), are by far the most studied gene family in RA. HLA-DR4 and more in particular the shared epitope (SE) are associated with the development of RA [6, 7]. The strongest associations with RA are found for genes in the HLA region, but recently also other genes were found to be associated with the development of RA (reviewed in [8]), such as the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene [9], the 6q23 region [10], the transducer and activator transcription 4 (STAT4) gene [11] and the genes TNF receptor associated factor 1 (TRAF1) and C5 [12].

However, twin studies showed that only approximately 60% of the development of RA can be explained by genetic factors [13]. Besides genetic factors, there are several environmental factors that might play a role in the pathogenesis of RA, such as smoking [14], infections [15] and hormonal factors (reviewed in [16]).

## Early arthritis

In RA, joint damage is irreversible and associated with a loss of functional capacity in a later stage of the disease [17, 18]. Early recognition and treatment is important, because with the current treatment it is possible to prevent radiographic progression [19, 20]. In most studies the diagnosis of RA is not made on clinical parameters, but patients have to fulfil the 1987 classification

criteria for RA of the American College of Rheumatology (ACR) [21]. Unfortunately, these classification criteria have a low sensitivity in early arthritis [22]. Therefore studies of early arthritis cohorts focus mostly on the prediction of radiographic progression, rather than on the presence or absence of RA according to the classification criteria of RA.

Many studies have focused on the prediction of radiographic progression in early arthritis patients. Presence of autoantibodies is the strongest determinant of radiographic progression in early arthritis. Especially antibodies against citrullinated proteins or peptides (anti-CCP) [23-25] and rheumatoid factor (RF) [26, 27] are predictors of radiographic progression early in the disease. Besides autoantibodies, genetic parameters [28] and parameters of disease activity, such as erythrocyte sedimentation rate (ESR) [28, 29], C-reactive protein (CRP) [30], the disease activity score [31] and quality of life [31] at the onset of disease are known to predict radiographic progression. Finally, there is a potential role of biomarkers of bone metabolism to predict radiographic damage at disease onset. Biomarkers such as C-terminal crosslink of type I collagen ( $\beta$ -CTX), Receptor Activator of Nuclear Factor Kappa B (NF $\kappa$ B) ligand (RANKL) and osteoprotegerin (OPG) are associated with future radiographic damage and could predict progression at the onset of disease [32-34].

In 36-54% of the cases, patients have radiographic damage shortly after the onset of symptoms [28, 35, 36]. Therefore, despite the improved early recognition of RA patients, half of the patients already have radiographic damage at the first visit to the rheumatologist. Since this damage is irreversible but potentially avoidable by modern treatment [19, 20], it is important to detect RA earlier, preferably before joint damage occurs.

## **Preclinical rheumatoid arthritis**

A major further step in the early recognition of RA would be to detect healthy persons at risk. To this end the preclinical phase of RA needs to be studied thoroughly. Little is known in this area as of yet. There are a number of possible methods to study preclinical RA. First, preclinical RA can be studied in a population with a high incidence of RA, such as Pima Indians [37] or unaffected first degree relatives from multicase RA families [38]. Second, specific cohorts can be used in which specimens were collected and blood samples of healthy people who developed RA later can be studied [39, 40].

Previous studies in preclinical RA patients mainly focused on autoimmunity and inflammation. RF and/or antikeratin antibodies were found in both high-risk [37, 38, 41] and healthy [39, 40, 42] populations before the start of the symptoms of RA. Autoantibody formation before the start of the symptoms was also described in several other autoimmune diseases, such as systemic lupus erythematosus [43] and insulin-dependent diabetes mellitus [44]. Results of preclinical inflammation studies in RA showed contradictory results [45, 46]. Aho et al did not find preclinical inflammation, measured by C-reactive protein (CRP) [45], which is in contrast with Masi et al who found a higher frequency of increased CRP levels in preclinical RA patients in comparison with healthy controls [46].

The limitation of these studies in preclinical RA patients was the fact that they were based on single serum samples. Therefore it was not possible to study the course of preclinical markers and to determine the moment of the appearance of these markers, which is necessary to make a reliable prediction of RA in healthy individuals or individuals at risk.

The studies described in this thesis are based on serial samples of blood donors who developed RA later. The studies were carried out with 79 RA patients and for each RA sample, 2 control samples were selected, matched for sex, age and time of blood donation to ensure identical storage conditions. Since the majority of the donors donate 2–4 times per year over periods of several years, a median of 13 serum samples per patient was available. These blood samples were collected, auto-antibodies were determined as well as genetic and clinical markers of the disease in order to predict factors of onset of RA and of radiographic damage in the future.

## **Thesis outline**

The purpose of this thesis is to study the preclinical phase of RA patients with serological markers by using serial blood samples of blood donors who developed RA later and of matched controls. These markers were used to predict the development of RA in the preclinical phase of the disease. Outcome data of the later patients were also used. In addition, the value of autoantibodies to predict RA and future radiographic damage in early arthritis was studied with data from the Early Arthritis Clinic (EAC) at the Jan van Breemen Institute. This EAC includes patients with early oligo- and polyarthritis (symptom duration less than 2 years).

Seven studies were done in preclinical RA patients. The presence of autoantibodies prior to disease and the predictive value of the antibodies for RA in healthy individuals at risk is presented in **chapter 2**.

Preclinical inflammation is investigated in two different studies. In the first study, CRP is measured and compared with healthy individuals, which is described in **chapter 3**. Another inflammatory marker, sPLA<sub>2</sub>, is used in combination with CRP and autoantibodies to study the development of the acute phase response and autoantibodies in preclinical RA in **chapter 4**.

In **chapter 5** measurements of total cholesterol, HDL cholesterol, triglycerides, apolipoprotein A-1, apolipoprotein B and lipoprotein (a) are used to study the lipid profile of preclinical RA patients.

In the Iowa Women's Health Study it was found that higher dietary intake of vitamin D (estimated by questionnaire) was associated with a lower risk of RA. We tested this possible association by directly measuring serum levels of 25-hydroxyvitamin D in preclinical RA (**chapter 6**).

To study early bone changes, markers of bone metabolism and regulators of osteoclast activity in preclinical RA patients were measured and associated with radiographic progression after the onset of RA in **chapter 7**.

The origin of RA is a combination of genetic and environmental factors. Therefore, genetic factors could be useful for the detection of RA in healthy individuals with an increased risk of RA. The association between autoantibodies (IgM-RF and anti-CCP) before the start of the symptoms of RA and genetic markers (HLA-DR4 and SE) is studied and the additional value of these genetic markers to predict RA in healthy persons is discussed in **chapter 8**.

After the emergence of clinically apparent arthritis, progression of disease can be studied in early arthritis patients. The diagnostic and prognostic value of anti-citrullinated fibrinogen in early arthritis are compared with IgM-RF and the second generation anti-CCP test in **chapter 9**.

A summary of the results, a general discussion and recommendations for future research are presented in **chapter 10**.

## References

1. Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME. Rheumatology. Third ed. Edinburgh: Mosby; 2003.
2. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005; 4(3):130-136.
3. Chorus AMJ. Reuma in Nederland: de cijfers. Actualisering 2000. TNO-rapport. 2001.
4. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. *Autoimmun Rev* 2005; 4(3):130-136.
5. Huizinga TW. Genetics in rheumatoid arthritis. *Best Pract Res Clin Rheumatol* 2003; 17(5):703-716.
6. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987; 30(11):1205-1213.
7. Nepom GT, Byers P, Seyfried C, Healey LA, Wilske KR, Stage D et al. HLA genes associated with rheumatoid arthritis. Identification of susceptibility alleles using specific oligonucleotide probes. *Arthritis Rheum* 1989; 32(1):15-21.
8. Bowes J, Barton A. Recent advances in the genetics of RA susceptibility. *Rheumatology (Oxford)*. 2008; 47(4):399-402.
9. Hinks A, Barton A, John S et al. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. *Arthritis Rheum* 2005;52:1694–9.
10. Thomson W, Barton A, Ke X et al. Rheumatoid arthritis association at 6q23. *Nat Genet* 2007;39:1431–3.
11. Remmers EF, Plenge RM, Lee AT et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007;357:977–86.
12. Plenge RM, Seielstad M, Padyukov L et al. TRAF1-C5 as a risk locus for rheumatoid arthritis – a genome-wide study. *N Engl J Med* 2007;357:1199–209.
13. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum* 2000; 43(1):30-37.
14. Heliövaara M, Aho K, Aromaa A, Knekt P, Reunanen A. Smoking and risk of rheumatoid arthritis. *J Rheumatol* 1993; 20(11):1830-1835.
15. Carty SM, Snowden N, Silman AJ. Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? *J Rheumatol* 2003; 30(3):425-429.
16. Masi AT, Aldag JC, Chatterton RT. Sex hormones and risks of rheumatoid arthritis and developmental or environmental influences. *Ann N Y Acad Sci* 2006; 1069:223-235.
17. Drossaers-Bakker KW, Kroon HM, Zwinderman AH, Breedveld FC, Hazes JM. Radiographic damage of large joints in long-term rheumatoid arthritis and its relation to function. *Rheumatology (Oxford)* 2000; 39(9):998-1003.

18. Kuper HH, van Leeuwen MA, van Riel PL, Prevoo ML, Houtman PM, Lolkema WF et al. Radiographic damage in large joints in early rheumatoid arthritis: relationship with radiographic damage in hands and feet, disease activity, and physical disability. *Br J Rheumatol* 1997; 36(8):855-860.
19. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. *Lancet* 1997; 350(9074):309-318.
20. Goekoop-Ruiterman YP, De Vries-Bouwstra JK, Allaart CF, van ZD, Kerstens PJ, Hazes JM et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. *Arthritis Rheum* 2005; 52(11):3381-3390.
21. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
22. Saraux A, Berthelot JM, Chales G, Le Henaff C, Thorel JB, Hoang S, et al. Ability of the American College of Rheumatology 1987 criteria to predict rheumatoid arthritis in patients with early arthritis and classification of these patients two years later. *Arthritis Rheum* 2001;44:2485-91
23. Kroot EJ, de Jong BA, van Leeuwen MA, Swinkels H, van den Hoogen FH, van't Hof M, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2000;43:1831-5.
24. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. *Arthritis Rheum* 2002;46:357-65.
25. Jansen LM, van Schaardenburg D, van der Horst-Bruinsma I, van der Stadt RJ, de Koning MH, Dijkmans BA. The predictive value of anti-cyclic citrullinated peptide antibodies in early arthritis. *J Rheumatol*. 2003 Aug;30(8):1691-5.
26. Eberhardt KB, Truedsson L, Pettersson H, Svensson B, Stigsson L, Eberhardt JL, Wollheim FA. Disease activity and joint damage progression in early rheumatoid arthritis: relation to IgG, IgA, and IgM rheumatoid factor. *Ann Rheum Dis*. 1990 Nov;49(11):906-9.
27. van der Heijde DM, van Leeuwen MA, van Riel PL, Koster AM, van 't Hof MA, van Rijswijk MH, et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;35:26-32.
28. Combe B, Dougados M, Goupille P, Cantagrel A, Eliaou JF, Sibilia J, Meyer O, Sany J, Daurès JP, Dubois A. Prognostic factors for radiographic damage in early rheumatoid arthritis: a multiparameter prospective study. *Arthritis Rheum*. 2001 Aug;44(8):1736-43.
29. Fex E, Jonsson K, Johnson U, Eberhardt K. Development of radiographic damage during the first 5-6 yr of rheumatoid arthritis. A prospective follow-up study of a Swedish cohort. *Br J Rheumatol*. 1996 Nov;35(11):1106-15.
30. Jansen LM, van der Horst-Bruinsma IE, van Schaardenburg D, Bezemer PD, Dijkmans BA. Predictors of radiographic joint damage in patients with early rheumatoid arthritis. *Ann Rheum Dis*. 2001 Oct;60(10):924-7.

31. Drossaers-Bakker KW, Zwinderman AH, Vlieland TP, Van Zeben D, Vos K, Breedveld FC, Hazes JM. Long-term outcome in rheumatoid arthritis: a simple algorithm of baseline parameters can predict radiographic damage, disability, and disease course at 12-year followup. *Arthritis Rheum*. 2002 Aug;47(4):383-90.
32. Landewé R, Geusens P, Boers M, van der Heijde D, Lems W, te Koppele J, van der Linden S, Garnero P. Markers for type II collagen breakdown predict the effect of disease-modifying treatment on long-term radiographic progression in patients with rheumatoid arthritis. *Arthritis Rheum*. 2004 May;50(5):1390-9.
33. Jansen LM, van der Horst-Bruinsma I, Lems WF, van Schaardenburg D, van de Stadt R, de Koning M, Dijkmans BA. Serological bone markers and joint damage in early polyarthritis. *J Rheumatol*. 2004 Aug;31(8):1491-6.
34. Geusens PP, Landewe RB, Garnero P, Chen D, Dunstan CR, Lems WF et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006; 54(6):1772-1777.
35. Hulsmans HMJ, Jacobs JWG, van der Heijde DMFM, Albada-Kuipers GA, Schenk Y, Bijlsma JWJ. The course of radiologic damage during the first six years of rheumatoid arthritis. *Arthritis Rheum* 2000;43:1927-40.
36. Bukhari M, Harrison B, Lunt M, Scott DGI, Symmons DPM, Silman AJ. Time to first occurrence of erosions in inflammatory polyarthritis: results from a prospective community-based study. *Arthritis Rheum* 2001;44:1248-53.
37. del Puente A, Knowler WC, Pettitt DJ, Bennett PH. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. *Arthritis Rheum* 1988; 31(10):1239-1244.
38. Silman AJ, Hennessy E, Ollier B. Incidence of rheumatoid arthritis in a genetically predisposed population. *Br J Rheumatol* 1992; 31(6):365-368.
39. Aho K, Heliövaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. *J Rheumatol* 1991; 18(9):1282-1284.
40. Halldorsdottir HD, Jonsson T, Thorsteinsson J, Valdimarsson H. A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. *Ann Rheum Dis* 2000; 59(2):149-151.
41. Walker DJ, Pound JD, Griffiths ID, Powell RJ. Rheumatoid factor tests in the diagnosis and prediction of rheumatoid arthritis. *Ann Rheum Dis* 1986; 45(8):684-690.
42. Kurki P, Aho K, Palosuo T, Heliövaara M. Immunopathology of rheumatoid arthritis. Antikeratin antibodies precede the clinical disease. *Arthritis Rheum* 1992; 35(8):914-917.
43. Aho K, Koskela P, Makitalo R, Heliövaara M, Palosuo T. Antinuclear antibodies heralding the onset of systemic lupus erythematosus. *J Rheumatol* 1992; 19(9):1377-1379.
44. Tuomilehto J, Zimmet P, Mackay IR, Koskela P, Vidgren G, Toivanen L et al. Antibodies to glutamic acid decarboxylase as predictors of insulin-dependent diabetes mellitus before clinical onset of disease. *Lancet* 1994; 343(8910):1383-1385.
45. Aho K, Palosuo T, Knekt P, Alha P, Aromaa A, Heliövaara M. Serum C-reactive protein does not predict rheumatoid arthritis. *J Rheumatol* 2000; 27(5):1136-1138.
46. Masi AT, Aldag JC, Sipes J. Do elevated levels of serum C-reactive protein predict rheumatoid arthritis in men: correlations with pre-RA status and baseline positive rheumatoid factors. *J Rheumatol* 2001; 28(10):2359-2361.

