Chapter 10

Summary and general discussion

Summary of the main findings

The purpose of this thesis was to study the preclinical phase of RA to find serological parameters that might help to predict the onset of RA. The studies were performed by using serial blood samples of blood donors who developed RA later. This blood donor pre-RA cohort with matched controls was the result of a collaboration between the Sanquin Blood Bank North West Region, which had stored serum from all blood donations since 1984, and the Jan van Breemen Institute (JBI), both located in Amsterdam, The Netherlands. At the JBI, a large regional rheumatology clinic, 79 RA patients were identified who had previously been blood donor. Of these, a median of 13 serum samples was available. Serological markers were used to predict the development of RA in the preclinical phase of the disease and to study pathogenetic events. Also, the value of autoantibodies to predict RA and future radiographic damage in early arthritis was studied with data from the Early Arthritis Clinic at the JBI.

Preclinical autoantibodies (Chapter 2)
In previous studies, autoantibodies have been demonstrated in serum samples from healthy subjects up to 10 years before they developed RA. However, the time course for the development of antibodies before onset of clinical RA is unknown, nor is it known which antibody, or combinations of antibodies, might be most sensitive or specific for predicting future development of the disease. Serum samples from pre-clinical RA patients were tested for IgM-RF and first-generation anti-CCP. Thirty-nine patients (49%) were positive for IgM-RF and/or anti-CCP on at least one occasion before the development of RA symptoms, a median of 4.5 years (range 0.1–13.8) before symptom onset. Of the 2,138 control samples, 1.1% was positive for IgM-RF, and 0.6% was positive for anti-CCP. The finding of an elevated serum level of IgM-RF or anti-CCP in a healthy individual implies an increased risk for the development of RA. IgM-RF and anti-CCP testing with appropriately high specificity may assist in the early detection of RA in high-risk populations.

Preclinical inflammation (Chapters 3 and 4)

Preclinical inflammation was investigated in two studies. In the first study, the presence of serologic signs of inflammation in patients with preclinical RA was investigated with serial measurements of highly sensitive CRP. For the periods 0–1 year, 1–2 years, and 4–5 years before the onset of symptoms, the median CRP concentration was increased in preclinical RA patients compared with controls. Furthermore, the CRP concentration increased significantly over time in patients with preclinical RA. The increase was most common within the 2 years before the onset of symptoms. The preclinical increase in CRP levels was observed both in donors with and without serologic abnormalities. The difference in CRP concentrations between preclinical RA patients and controls may be a significant factor in the development of later symptomatic inflammation. However, there were only small differences in CRP levels between preclinical RA patients and controls. Therefore, the findings of this study can be used only in a population of patients and are not suitable for decision-making in individual patient care.

In the second study, the temporal relationship between onset of inflammation (measured by sPLA2 and CRP) and the presence of autoantibodies (IgM-RF and anti-CCP) was investigated in preclinical RA. IgM-RF and anti-CCP concentrations were significantly associated ($p<0.001$) with concentrations of sPLA2, CRP, and the combination of sPLA2 and CRP at the same time point. However, we found no stronger association between the two autoantibody tests and the three inflammation measures 1, 2, and 3 years before or after a time point than for measurements at the same time, in the whole group or in subgroups of IgM-RF and anti-CCP positive patients. In conclusion, both the acute phase response and autoantibody formation often develop years before the first symptoms of RA. These phenomena are probably closely connected in time.

Preclinical lipid profile (Chapter 5)

RA is characterised both by inflammation and an increased cardiovascular risk. Active early RA is associated with dyslipidaemia, which may partially explain the enhanced cardiovascular risk. However, it is unknown when this dyslipidaemia starts. Therefore, levels of total cholesterol, HDLc, triglycerides, apo AI, apo B and Lp(a) were determined in 1078 serum samples of 79 blood donors who later developed RA and compared with 1071 control samples, matched for age, sex and storage time. Samples of patients who later developed RA showed, on average, 4% higher total cholesterol, 9% lower HDLc, 17% higher triglyceride and 6% higher apo B levels than matched controls ($p< 0.05$). The magnitude of

the differences in lipid levels between groups explained by CRP was limited. It was concluded that patients who later develop RA have a considerably more atherogenic lipid profile than matched blood donors for at least 10 years before onset of symptoms.

Preclinical vitamin D levels (Chapter 6)

It was recently reported that higher dietary intake of vitamin D as measured by questionnaire was associated with a lower risk of RA. However, it is well-known that sun exposure is a much more important source of vitamin D than dietary intake. Since the total body amount of vitamin D can be accurately estimated by serum levels of 25-hydroxyvitamin D (25(OH)D), we tested the hypothesis that 25(OH)D serum levels of blood donors who developed RA later would be lower than matched controls. From each patient serum samples were selected from the time points 1 year, 2 years and 5 years or longer before the start of the symptoms, respectively, together with one control donor sample per patient sample. At all time points there was no association between vitamin D deficiency and later RA. The geometric mean 25(OH)D concentration was slightly lower in the patients compared with the controls (29.8 vs. 32.1 nmol/l), but the difference was not statistically significant. It was concluded that there is no difference between 25(OH)D serum levels in patients who later develop RA and healthy donors, which suggests that vitamin D does not play an important role in the pathogenesis of RA.

Preclinical bone markers and regulators of osteoclast activity (chapter 7)

This chapter describes a study in which it was tested whether the presence of autoimmunity and inflammation in preclinical RA is accompanied by alterations in bone metabolism. In preclinical RA patients and controls, the following markers were measured: 1) markers for bone formation: osteocalcin (OC) and N-terminal propeptide of type I collagen (P1NP), 2) a marker of bone resorption: β-C-telopeptide (β-CTX), and 3) regulators of osteoclast activity: receptor activator of NFκB ligand (RANKL) and osteoprotegerin (OPG). Correcting for age, gender, time of blood donation, autoantibodies and inflammation, the group of preclinical RA patients had increased mean levels of P1NP and OPG compared with the control group. Preclinical levels of P1NP and OPG were negatively associated with radiographic progression after the onset of the symptoms of RA, but these associations were not statistically significant. It appears that the presymptomatic phase of RA is characterized not only by autoimmunity and increased inflammation, but also by a parallel alteration of bone metabolism.

HLA-DR4 and Shared Epitope in preclinical RA (Chapter 8)
The association between genetic markers (HLA-DR4, now mostly measured as the shared epitope, SE) and the presence of autoantibodies (IgM-RF and anti-CCP) was determined in preclinical RA. Of the 56 preclinical RA patients of whom DNA was available, 26 were positive for anti-CCP (46%), 13 for IgM-RF (23%), 32 for HLA-DR4 (57%) and 47 for SE (84%). Anti-CCP was significantly associated with the presence of HLA-DR4 (p=0.03, OR: 3.5; 95% CI 1.1-11.0). However, the association between anti-CCP and SE did not reach significance (p=0.11, OR: 3.7; 95% CI 0.7-19.4), probably due to the small number of patients. IgM-RF was not statistically significantly associated with HLA-DR4 (p=0.31, OR: 2.0; 95% CI 0.5-7.3) and the association with SE did just not reach significance (p=0.07, OR could not be calculated due to the absence of patients with the combination IgM-RF+ and SE-). In conclusion, anti-CCP and carriership of HLA-DR4 and the SE allele are positively associated in preclinical RA. Since there was no DNA available of controls, it was not possible to use HLA-DR4 and SE for a risk calculation for the development of RA in the preclinical phase of the disease.

Autoantibodies in early arthritis (Chapter 9)
The anti-CCP test has a high sensitivity and specificity for RA, although CCP is not the physiological target of the autoantibodies. Citrullinated fibrin is abundant in inflamed synovium. The objective of this study was to assess the diagnostic and prognostic value of anti-citrullinated fibrinogen (ACF), a soluble precursor of fibrin, in comparison with IgM-RF and the second generation anti-CCP test. The sensitivities of the ACF, anti-CCP, and IgM-RF tests were 55.8%, 57.8%, and 44.6%, with specificities of 92.6%, 94.2%, and 96.7%, respectively. Approximately 30% of the IgM-RF negative patients were positive for ACF or anti-CCP or both. The ACF and anti-CCP test had a high agreement in early arthritis (kappa = 0.84). Of all baseline characteristics, the ACF test and the anti-CCP test were the best predictors for diagnosing RA at one year (OR = 10.3 and 10.6, respectively) and for radiographic progression after two years (OR = 12.1 and 14.8). This study shows that ACF is as sensitive as anti-CCP and more sensitive than IgM-RF in diagnosing rheumatoid arthritis in early arthritis. The ACF test is also a good predictor of radiographic progression, with a performance similar to the anti-CCP test. The ACF test and the anti-CCP test are especially valuable in IgM-RF negative arthritis.

Discussion

Based on the findings of the different studies in this thesis, it can be concluded that several processes start years before the onset of the symptoms of RA in many patients, which eventually lead to the development of clinical disease. Interaction of genetic and environmental factors results in the production of antibodies (rheumatoid factors and various types of anti citrullinated protein antibodies (ACPA)), inflammatory mediators such as CRP and sPLA2, alterations of bone metabolism and dyslipidemia before disease onset. The research design made it possible to determine the time course of several serum markers in preclinical RA patients. What do these findings mean in relation to the aetiology of RA? And what are the possible consequences of the results described in this thesis for the prediction of RA in healthy populations and populations at risk?

Aetiology of rheumatoid arthritis

The genetic base of RA is estimated to be 60% [1]. Therefore, environmental factors must also play an important role. Clinically apparent disease is preceded by a steady increase in inflammatory activity during a few years, accompanied by elevations of various cytokines [2, 3]. Alterations in bone metabolism occur in the same period and are probably a direct result of the inflammation. The inflammation itself in (preclinical) RA is an aspecific phenomenon, similar to other inflammatory disease states. The most specific characteristic of RA, the presence of ACPA, occurs on average before the elevation of RF and in this study up to 14 years before the first symptoms. This makes ACPA a likely candidate as a key player in the pathogenesis of at least the more severe forms of the disease, more so than RF.

Recent evidence points to an interaction between the genetic makeup, notably the shared epitope, and ACPA [4]. Less strong associations with RA have been found for the PTPN22 [5], STAT4 [6] and TRAF1/C5 [7] polymorphisms. The physiologic basis of the latter genes in disease susceptibility is not yet fully clear. Of interest, the finding that dyslipidemia is present at least 10 years before the symptoms, points to either a shared genetic background of RA and dyslipidemia, or to longstanding unhealthy lifestyle factors in the later patients. Either way, the wellknown increased risk of cardiovascular disease in RA patients may well originate before the disease becomes manifest.

Indeed, the long duration of the preclinical period suggests that environmental factors must have a prolonged or repeated influence on a situation with an

immunological imbalance causing a chronic and increasing inflammation. In this respect, especially the combination of smoking and SE presence have been implicated [8]. Tobacco smoke would produce inflammation and citrullination in the lung in SE positive individuals. The next intensification step would be the break of tolerance to citrullinated antigens with ensuing systemic autoimmunity and possibly activation of inflammation at other sites of citrullination, such as the joints [9]. Unhealthy dietary habits could come into the play by favouring a background state of low grade inflammation, as the blood of preclinical RA patients was shown to contain on average lower concentrations of several antioxidants [10]. Viral infections may also contribute, again probably in an unspecific manner by inducing a pro-inflammatory state, since no single infection has been reliably related to the development of RA. To conclude this short discussion of environmental and genetic risk factors, an association was found between periodontal disease and RA (reviewed in [11]). This suggests either a specific effect of the involved micro organism *Porphyromonas gingivalis*, which is capable of inducing citrullination, or risk-enhancement by the systemic effect of the local inflammatory state [12].

Limitations of the research design

The preclinical phase of RA is difficult to study, because the affected individuals have no complaints yet and therefore do not seek medical attention. Given the low incidence of RA (0.2-0.4 per 1000 per year [13]), prospective studies in healthy individuals are impossible, because for a sufficient number of cases several years follow-up are necessary in an extremely large study population. Therefore, alternative research designs are necessary to study the preclinical phase, such as the use of a high risk population (examples: multi-case families [14] or Pima Indians [15]), or the use of stored preclinical serum samples of a RA cohort [16, 17].

With the latter alternative research design, the existence of a preclinical phase has been clearly demonstrated. However, the number of patients and the number of serum samples in these studies were limited as a result of which the course of preclinical markers and determination of the start of the appearance of these markers was unknown. Thus a reliable prediction of RA in healthy individuals or individuals at risk becomes difficult. The present research design had the advantage of often multiple available samples. Still, blood donors donate blood only 3-4 times per year, as a result of which studying the role of intercurrent infections is difficult, because the chance of finding evidence of short infectious episodes in the sera is very small.

The used research design also has other disadvantages. First, the time of the start of the complaints was determined by chart review. Since patients can have complaints for a long period at the first visit to the rheumatologist [18], it is difficult to define the exact moment of the start of the complaints. However, it is unlikely that this has biased the results of the studies in this thesis, since RA has a long preclinical phase. Secondly, additional information about lifestyle, such as smoking and nutrition, and menopausal status of the females was lacking. In the patient group it was possible to obtain this information from chart review, but the controls were anonymous. Therefore, statistical analyses could not be corrected for these parameters. Since the sera of patients and controls were matched on age, gender and time of blood donation, it is not expected that this will have influenced differences between patients and controls. Finally, only serum samples were available, as a result of which other relevant parameters, such as genetic profiling, could not be performed in all patients despite additional efforts to extract DNA from peripheral blood cells from the serum.
In the future, the present design should be complemented by the study of prospective cohorts of individuals at risk.

Prevention of RA in healthy individuals
RA management has become focussed on detecting and treating RA as early as possible in order to prevent structural joint damage, a situation which is called secondary prevention. The results from this thesis can be used for the development of primary prevention of RA, i.e. prevention of the development of RA in healthy individuals.
Before primary prevention of RA is possible, it is important that people at risk can be traced by using one or more tests. Of all measured parameters in this thesis, only the presence of antibodies, particularly ACPA, can be used to predict future RA in healthy individuals. None of the other preclinical parameters appeared to be suitable for predicting RA, because differences between patients and controls were too small. The additional value of genetic factors is unclear, since DNA was not available of the controls. In a recent study, It was found that SE in combination with a positive anti-CCP increased the risk of future RA [19], but the number of used controls was to low to make a reliable estimate of the risk. Given the high association between SE and ACPA, it is plausible that a genetic measurement will not have additional value above the anti-CCP test for calculating the risk on RA. To test the additional value of genetic parameters, the association between genetic factors and ACPA in healthy individuals has to be studied.

However, the predictive value of ACPA in a healthy population is limited. The risk of developing RA within five years for healthy persons with a positive anti-CCP test was estimated at 5%. To raise the predictive value of a positive test result, the test must be carried out in a healthy population at risk. For this purpose, healthy first degree family members of RA patients could be useful, since in these individuals the risk of developing RA increases to 70%. The prevalence of RA is about 1% in the Netherlands [20], resulting in approximately 150,000 RA patients with varying numbers of family members. This large group of healthy individuals at risk can serve as a research population for the improvement of predicting future RA in healthy individuals. When predicting future RA in the healthy population is possible, this can result in population-based screening of individuals at risk and subsequently the testing of preventive treatment.

Preventing RA in arthralgia patients

A related approach is to try to prevent the development of RA in patients with arthralgia. Arthralgia patients do not have arthritis by clinical standard, but experience painful and stiff joints, which may be the first symptoms of RA. It is likely that these patients have an increased risk of developing RA, although the exact incidence of RA in this patient group is unknown. Therefore, it is plausible that also in this group RA specific antibodies have a good predictive value for the future development of RA. General practitioners (GP's) will play a vital role in the detection of these patients at risk, since GP's have a gatekeeper role for access to specialized care in the Netherlands and the GP is the first professional to be consulted for health problems. To optimise possible prevention of RA in this stage of the disease, the GP should distinguish arthralgia of hands and feet with morning stiffness ("inflammatory arthralgia") from other causes of joint pain, test these patients for ACPA and IgM-RF and refer patients with a positive test result to a rheumatologist [21].

Conclusion

The results from this thesis can be used for the development of primary prevention of RA, i.e. prevention of RA development in "healthy" individuals without arthritis. Of all measured parameters in this thesis, only the presence of antibodies, particularly ACPA, can be used to predict future RA in healthy individuals. A prevention strategy of RA in individuals without arthritis seems to be possible only in high risk populations such as persons with first degree family members with RA or patients with arthralgia. Research in these persons at risk will result in a better understanding of the aetiology of the disease and improvement of the prediction of RA in individuals without arthritis. When these

future RA patients can be traced and a treatment can be found to postpone or prevent the development of RA, this will improve the quality of life of a large number of future RA patients as well as reduce the high medical and societal costs associated with this disease.

References

1. 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.
2. Holers VM. Antibodies to citrullinated proteins: pathogenic and diagnostic significance. Curr Rheumatol Rep. 2007 Oct;9(5):396-400.
3. Jørgensen KT, Wiik A, Pedersen M, Hedegaard CJ, Vestergaard BF, Gislefoss RE, et al. Cytokines, autoantibodies and viral antibodies in premorbid and postdiagnostic sera from patients with rheumatoid arthritis: case-control study nested in a cohort of Norwegian blood donors. Ann Rheum Dis. 2008 Jun;67(6):860-6.
4. Kaltenhäuser S, Pierer M, Arnold S, Kamprad M, Baerwald C, Häntzschel H et al. Antibodies against cyclic citrullinated peptide are associated with the DRB1 shared epitope and predict joint erosion in rheumatoid arthritis. Rheumatology (Oxford). 2007 Jan;46(1):100-4.
5. Hinks A, Barton A, John S, Bruce I, Hawkins C, Griffiths CE, 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.
6. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 2007;357:977–86.
7. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis – a genomewide study. N Engl J Med 2007;357:1199–209.
8. Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006 Jan;54(1):38-46.
9. Klareskog L, Rönnelid J, Lundberg K, Padyukov L, Alfredsson L. Immunity to citrullinated proteins in rheumatoid arthritis. Annu Rev Immunol. 2008;26: 651-75.
10 Heliövaara M, Knekt P, Aho K, Aaran RK, Alfthan G, Aromaa A. Serum antioxidants and risk of rheumatoid arthritis. Ann Rheum Dis. 1994 Jan;53(1):51-3.
11. Mercado FB, Marshall RI, Bartold PM. Inter-relationships between rheumatoid arthritis and periodontal disease. A review. J Clin Periodontol. 2003 Sep;30(9):761-72.
12. Marotte H, Farge P, Gaudin P, Alexandre C, Mougin B, Miossec P. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. Ann Rheum Dis 2006; 65:905–9
13. Chorus AMJ. Reuma in Nederland: de cijfers. Actualisering 2000. TNO-rapport. 2001.
14. Silman AJ, Hennessy E, Ollier B. Incidence of rheumatoid arthritis in a genetically predisposed population. Br J Rheumatol 1992; 31(6):365-368.
15. 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.

16. Aho K, Heliovaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. J Rheumatol 1991; 18(9):1282-1284.

17. 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.

18. Hernández-García C, Vargas E, Abásolo L, Lajas C, Bellajdell B, Morado IC, Macarrón P et al. Lag time between onset of symptoms and access to rheumatology care and DMARD therapy in a cohort of patients with rheumatoid arthritis. J Rheumatol. 2000 Oct;27(10):2323-8.

19. Berglin E, Padyukov L, Sundin U, Hallmans G, Stenlund H, Van Venrooij et al. A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis. Arthritis Res Ther. 2004;6(4):R303-8.

20. Chorus AMJ. Reuma in Nederland: de cijfers. Actualisering 2000. TNO-rapport. 2001.

21. Van Schaardenburg D, Van der Horst-Bruinsma IE. Reumatoïde artritis nog vroeger herkennen en behandelen? Huisarts Wet 2007;50(1):16-8.