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.