



Summary and discussion

In the first part of this thesis investigations are reported on generalized bone loss in patients with rheumatoid arthritis. In the second part, several studies are described that we performed in our cohort of patients with rheumatoid arthritis (RA) treated with infliximab, a TNF α -blocking agent.

PART I

In **chapter 2** data are reported on the effects of infliximab on bone and bone metabolism in patients with RA. In **chapter 2A** the short-term effects of infliximab on bone metabolism in a cohort of 68 RA patients are shown. In this study bone formation was measured by osteocalcin (OC), N-terminal peptide of type 1 procollagen (PINP) and bone specific alkaline phosphatase (BALP), whereas bone resorption was measured by β -isomerized carboxy terminal telopeptide of type 1 collagen (β -CTX) and carboxy terminal peptide of type I collagen (ICTP). Both OC and PINP showed a significant increase and β -CTX a significant decrease during 14 weeks of treatment with infliximab. These changes in bone resorption (β -CTX) were correlated with the change in disease activity (DAS-28).

In **chapter 2B** data are presented on the effect of 1 year of treatment with infliximab on BMD (spine, hips and hands) and markers of bone metabolism in a cohort of 102 RA patients. In this study, treatment with infliximab arrested generalized bone loss in hips and lumbar spine, whereas localized bone loss in hands, measured with digital X-ray radiogrammetry (DXR), persisted (-0.8%, $p < 0.01$). In patients with a good clinical response to treatment defined by the EULAR response criteria, there was an increase of BMD in hip and spine, whereas there was a decrease of BMD in patients who had a less favourable clinical response. This relationship between disease activity and bone loss was further supported by the correlation between change in DAS-28 and CRP with β -CTX. Receptor activator of NF κ b ligand (RANKL), an activator of osteoclast differentiation and activation, decreased significantly whereas osteoprotegerin (OPG) remained stable, leading to a favourable change in its ratio.

In conclusion, treatment with infliximab reduces bone resorption whereas it increases bone formation in patients with rheumatoid arthritis, subsequently arresting generalized bone loss. However, localized bone loss in hands persists.

Chapter 3 describes the changes of BMD in spine, hip and hands and the occurrence of fractures in patients with rheumatoid arthritis over a period of 5 years. This project was performed by the OSTRAGroup. Five years ago the OSTRAGroup established a cohort of 150 female RA patients with an established disease (> 5 years). The main result of the baseline study was that a low BMD in hip and vertebral fractures was associated with radiological damage of the joints (total Larsen-score), in addition to

other well-known determinants: high age, low BMI, and high cumulative doses of corticosteroids.

In total, 102 out of these 150 patients were included in the 5-year follow-up study. **Chapter 3a** describes the fractures that occurred during the follow-up period. It shows that there was a high rate of vertebral and non-vertebral fractures during those 5 years. A total of 18 out of 102 patients (17.6%) sustained a new non-vertebral fracture during follow-up and in 18 out of 97 patients (18.5%) new vertebral fractures were identified on spinal X-rays. This resulted in an annual incidence rate of 3.2 (95% CI 1.8–5.5) per 100 patients years for non-vertebral fractures and of 3.7 (95% CI 2.2–5.8) per 100 patients years for morphometric vertebral fractures. These incidence rates are higher than those found in general population in individuals of the same age and gender: 1.9/100 patient years and 0.8 to 1.0/100 patient years respectively [1-4].

Furthermore, a substantial decrease in BMD was observed in hip and spine during the follow-up period (**chapter 3b**). There was a significant decrease of 5.9% ($p < 0.05$) in total-hip BMD (g/cm^2) and in spine (L2–L4) there was a trend towards a loss (-2.4%, $p = 0.059$). Again, this is higher than the rate of BMD loss found in general population. The loss of cortical hand BMD measured by DXR from hand radiographs, is described in **chapter 3b**. The mean (95% CI) DXR-BMD change was -6.7% (-11.2, -2.82%). In this study high disease activity at baseline measured by DAS-28 was an independent predictor for cortical hand bone loss over a period of 5 years.

The conclusions of the studies described in **chapter 3** show a substantial amount of bone loss and a high risk of fractures in postmenopausal women with RA. This is remarkable, since modern anti-rheumatic therapies like TNF blocking agents and effective anti-osteoporotic drugs, such as bisphosphonates, are widely available. This implicates that rheumatologists need to pay more attention to osteoporosis when treating RA patients, by aiming at disease remission and using bisphosphonates more frequently.

PART II

In the second part of this thesis data are presented on several studies undertaken in our cohort of infliximab-treated RA patients. This cohort was established at the Jan van Breemen Institute, Slotervaart hospital and VU University medical center (all situated in Amsterdam, the Netherlands) in 2001, at the start of the introduction of infliximab in the Netherlands. All consecutive patients treated with infliximab were included into this cohort. Patients were followed for disease activity and side effects, blood samples were taken at each visit and X-rays and a DEXA-scan were performed yearly.

Patients with RA have an increased risk of morbidity and mortality compared to general population. Mortality due to cardiovascular disease (CVD) is the main cause of death in patients with RA. This excess of cardiovascular mortality in patients with RA is predominantly due to accelerated atherosclerosis, possibly induced by inflammation in RA. There is evidence that inflammation in RA is associated with a worsening of the lipid profile [5, 6].

In **chapter 4a** data are presented on the short-term effect of infliximab treatment on lipid profiles during the first 14 weeks. It was found that treatment with infliximab led to a significant increase of both total- and HDL-cholesterol levels 0.4mmol/l and 0.1mmol/l, respectively. These changes were inversely related to the change in disease activity. However, the atherogenic index (ratio between total- and HDL-cholesterol), an important prognostic indicator for future CVD, remained constant.

Chapter 4b describes the long-term effect of infliximab on lipid levels in RA patients. In this study we observed a significant increase of total- and HDL-cholesterol levels after 6 weeks of infliximab treatment, which gradually returned to baseline after 48 weeks. Longitudinal analyses revealed significant, yet opposite, associations between lipid levels and disease activity and between lipid levels and prednisone dose. DAS-28 improvement by 1 point is associated with an increase of 0.016 mmol/l (0.618 mg/dl) total- and 0.045 mmol/l (1.737 mg/dl) HDL-cholesterol. Reduction of 10 mg of prednisone is associated with a decrease of 0.04 mmol/l (1.544 mg/dl) total- and 0.16 mmol/l (6.177 mg/dl) HDL-cholesterol. The initial beneficial effect of infliximab on the lipid profile, a reduction of disease activity, seems to be attenuated by a concomitant decrease in prednisone dose.

Chapter 4 implicates that the inflammation in RA leads to changes in cholesterol, although the reduction of inflammation by infliximab does not directly lead to an improvement in lipid profile (no change in atherogenic index).

One of the characteristics of RA is the presence of auto-antibodies. IgM-RF is observed in about 75% of RA patients, but it is also frequently observed in other inflammatory diseases. Antibodies against cyclic citrullinated peptide (anti-CCP) target citrullinated proteins and are highly specific for RA and present in about 80% of RA patients [7, 8].

These antibodies are also found years before the onset of the disease. That is why anti-CCP is often considered as indicators for the pathogenesis of RA.

Chapter 5 shows that in our cohort of RA patients treated with infliximab over a period of one year, both IgM-RF and anti-CCP had decreased significantly. Antibodies against deiminated fibrinogen, a specific citrullinated peptide that is found in inflamed joints of RA patients, also decreased significantly. In terms of percentages, the levels of IgM-RF were reduced by 64%, whereas the anti-CCP and ACF levels were reduced

by roughly 25%. ACF levels showed a large decrease in patients with a short disease duration. These data would imply that if auto-antibodies in RA really have a pathogenic role, that treatment with infliximab, especially early treatment, could alter the onset of the disease.

Treatment with infliximab provides great benefit for many patients with rheumatoid arthritis. However, some patients have a persistent active disease whereas others show loss of efficacy after prolonged treatment. Infliximab, which is a chimeric monoclonal antibody (partly human, partly mouse), may induce IgG-auto-antibodies that may cause loss of efficacy and may lead to allergic reactions [9, 10]. The results presented in **chapter 6** show that antibodies against infliximab were detected in 22 patients (43%). Patients with anti-infliximab antibodies were significantly more often classified as non-responders to treatment, compared to patients without detectable anti-infliximab antibodies (20/29 69%; versus 8/22 (36%), $p=0.04$). In addition, anti-infliximab antibodies could be detected in all patients with an allergic reaction ($n=3$). In conclusion, treatment with infliximab may induce anti-infliximab antibodies, which may lead to loss of efficacy and may cause allergic reactions to infliximab.

Infliximab is considered to be relatively safe in randomized controlled trials for the registration of the drug [11]. However, this could be different in daily clinical practice, because of patient selection in randomized clinical trials. That is why we determined the adverse events, in particular infections, in RA patients treated with infliximab in daily clinical practice, who usually have more co-morbidity and co-medications than trial patients. These findings are reported in **chapter 7**. Patients frequently had infections (43–57%, depending on the definition used), mostly on the upper respiratory tract and the lower urinary tract. The incidence of serious infections was 0.08/patient-year. These numbers are comparable to the infection rates found in randomized trials, indicating that infliximab can be safely administered in daily clinical practice.

Gastro-intestinal complaints are the most common side effects of oral bisphosphonates. Therefore, pamidronate can be an attractive alternative for those patients who do not tolerate oral bisphosphonates, or for those who have a severe contra-indication for them [12, 13]. In **chapter 8** the changes in BMD are described during one year of treatment with intravenous pamidronate (60 mg every 3 months) in patients who do not tolerate oral bisphosphonates, compared to patients who do and who are treated with alendronate. This study shows that in patients visiting our osteoporosis clinic, treatment with intravenous pamidronate was as effective as treatment with oral alendronate, measured by the effect on BMD. The BMD of lumbar spine increased by 4.0% ($p<0.05$ vs baseline) in both groups, and the BMD of hip increased by 3.3% and 2.9% ($p<0.05$ vs baseline) in the alendronate and pamidronate groups, respectively.

DISCUSSION

Generalized bone loss is an important extra-articular of rheumatoid arthritis. In our OSTRACOHORT, patients were treated according to modern treatment concepts, including the use of TNF-blockers and combination therapy, however, there was still a high rate of bone loss. BMD in spine and hips decreased significantly and there was a high incidence of vertebral and non-vertebral fractures: one fifth of the female patients with established RA suffered a vertebral fracture and another 19 percent a non-vertebral fracture during a 5 years observational period. These fractures will contribute to a decrease in quality of life for these patients. Effective anti-osteoporotic therapies are available for the prevention of fractures. It is therefore important for rheumatologists to focus on the prevention of generalized bone loss, particularly in those patients who are at high risk of fractures: elderly women, prednisone users, and patients with a very active disease.

Furthermore, treatment with a TNF-blocker seems to arrest generalized bone loss in RA patients, but it does not arrest localized bone loss. We established a relation between disease activity and generalized bone loss. This could implicate that, with the new anti-rheumatic treatment strategies and biologicals, less osteoporosis will be observed in RA patients. However, continuing bone loss in hands during effective anti-rheumatic treatment could also imply that inflammatory control alone is not sufficient. It may be necessary to add adjuvant bone protection to anti-inflammatory treatment. Bone loss in rheumatoid arthritis is characterized by an increase of bone resorption. Bisphosphonates are potent inhibitors of osteoclast activity and could therefore be an attractive candidate for such an adjuvant therapy. There is already evidence that bisphosphonates are effective in the prevention of erosions in animal and human studies [14, 15]. Another interesting drug could be denosumab, a biological that blocks RANKL and, consequently osteoclast differentiation and activation. In this thesis we have shown that there is increased expression of RANKL in active RA. Other studies have shown increased levels of RANKL in inflamed joints of RA patients. Also blocking RANKL in animal models has prevented joint erosions without influencing inflammation [16]. A pilot study in RA patients showed similar but slightly disappointing results [17]. There is also some evidence that the Wnt signalling pathway is involved in the repair of erosions. Blocking antagonists of Wnt, such as sclerostin and dickopf1, with antibodies prevents the development of erosions in experimental animal models of rheumatoid arthritis [18]. However, future investigations are needed to clarify the relationship between local bone loss and inflammation and to determine whether adjuvant therapies could be beneficial for RA patients.

The studies in this thesis are cohort studies without appropriate control groups, which makes it hard to draw definite conclusions from them. However they raise questions for further research to expand and elucidate the results.

Most of the studies in part two have led to further investigations within our department. This is especially true for example for the implication of antibodies against infliximab in the loss of efficacy of infliximab during treatment. These neutralizing antibodies are also detected in patients with other rheumatic diseases than RA (psoriatic arthritis and ankylosing spondylitis and they also lead to loss of efficacy in these patient groups [19, 20]. New assays were developed to detect auto-antibodies for other biologicals. (adalimumab and rituximab) Further studies have also shown that patients who developed auto-antibodies for infliximab could successfully be switched to adalimumab. These patients even responded better to adalimumab than patients who had an initial low response to infliximab [21].

Determination of antibodies against TNF blockers is now frequently used to decide about continuation of therapy. However, future studies are needed to further clarify the clinical use of these antibodies.

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