Rheumatoid arthritis: biologicals and bone

M. Vis

2011

Cover design Kitty van der Veer, Proefschrift.nu
Layout Renate Siebes, Proefschrift.nu
Printed by Labor Grafimedia BV, Utrecht
ISBN 978-90-9026026-6

The printing of this thesis was supported by Eli Lilly, Roche, UCB Pharma B.V., Amgen B.V., Novartis Pharma B.V., Abbot B.V., MSD B.V., and AstraZeneca.

VRIJE UNIVERSITEIT

Rheumatoid arthritis: biologicals and bone

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op dinsdag 5 april 2011 om 15.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Marinus Vis

geboren te Utrecht

promotoren:	prof.dr. W.F. Lems prof.dr. B.A.C. Dijkmans
copromotoren:	dr. A.E. Voskuyl dr. G.J. Wolbink

Voor mijn vader

Contents

PART II

Chapter 1

Introduction and outline of the thesis

INTRODUCTION

This thesis describes several aspects of rheumatoid arthritis (RA) and osteoporosis and the link between them. The introduction of this thesis gives a description of both diseases and it addresses several aspects of the diseases that we investigated.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic systemic inflammatory disease that may affect many tissues and organs but that mainly affects the joints. RA is characterized by synovitis and destruction of the small joints of the hands and feet. The disease affects approximately 0.5 to 1 percent of the Caucasian population and it has a peak incidence between the ages of 50 and 60 [1-5]. One of the other characteristics of RA is the presence of auto-antibodies: IgM rheumatoid factor (IgM-RF) and anti-citrullinated protein antibodies (anti-CCP).

The aetiology of RA remains unknown. There is, however, increasing evidence that anti-CCP plays a role in the development of RA. Citrullinated proteins are found in inflamed joints of RA and also, to a much lesser extent, in non-RA patients but antibodies against these proteins are highly specific only for RA patients. These antibodies can be detected in the serum of patients years before the development of the disease [7]. These findings strongly suggest a pathological role for the antibody response in the development of RA [8, 9].

RA can be a disabling and painful condition, which can lead to a substantial loss of function and mobility with an increased risk of morbidity and mortality [10-13]. This increased mortality is mainly due to an increase of deaths caused by cardiovascular diseases (CVD) [14, 15]. Another associated co-morbidity is osteoporosis: patients who tend to have a low BMD with an increased fracture risk. Inflammation, use of steroids and immobility seem to be the main risk factors.

Disease Modifying Anti-rheumatic Drugs (DMARDs) are the choice for treatment of patients with rheumatoid arthritis. The ultimate goal for treatment is to achieve drug-free remission. Remission is defined as a state with no or little disease activity, no progression of joint destruction and no further loss of quality of life [16]. Treatment of RA has improved dramatically over the past decade. It has changed from a "start-low-go-slow" approach into a strategy of early intervention with more aggressive (combination) strategies and close monitoring aiming to achieve remission as soon as possible. This new approach is highly effective in controlling disease activity and preventing joint damage [17-19].

The introduction of the so-called biologicals is another big improvement in the treatment of RA. These drugs are made through biotechnology and they target specific

mediators in the inflammation process. Currently, biologicals are available against several pro-inflammatory cytokines (Il-1, TNFα and IL-6) and against B-cells and activation of T-cells. Biologicals against the pro-inflammatory cytokine Tumor Necrosis Factor (TNFα) were the first biologicals, introduced for use in clinical practice.

TNF blockers have shown to be able to control disease activity effectively and to reduce joint destruction, particularly when given in combination with methotrexate [20-23]. However, because of the powerful immune suppression by these biologicals there is an increased risk of infections (number and severity) during treatment, especially reactivation of tuberculosis [24, 25]. Another issue is that a number of patients with rheumatoid arthritis may lose their initial response to biologicals [26]. One of the possible reasons for this could be the formation of neutralizing antibodies against these drugs (human anti-human antibodies (HAHA) and human anti-chimeric antibodies (HACA)) [27].

Osteoporosis

Osteoporosis is a disease, defined as loss of bone mineral density and a deterioration of micro-architecture leading to an increased risk of fractures [28]. Osteoporosis predominantly occurs in postmenopausal women: it is related to a lower peak bone mass in women and accelerated bone loss after menopause. Furthermore, life expectancy for women is higher than for men, which is also an important reason for a high fracture rate in women, because the incidence of fractures increases with age [29]. Fractures most frequently occurring in osteoporosis are wrist, hip and vertebral fractures [30, 31]. These fractures, especially hip fractures, have a major impact on quality of live and also on mortality rates [30, 32].

The diagnosis of osteoporosis is made by measuring bone mineral density (BMD) of hip or spine. BMD is measured using a dual X-ray absorptiometry machine (DXA). Osteoporosis is diagnosed when the bone mineral density is less than or equal to 2.5 standard deviations in spine and/or hips, which is below the BMD of a young adult reference population. This is translated as a T-score. The World Health Organization has established the following diagnostic guidelines [33]:

- T-score -1.0 or greater is “normal”
- T-score between -1.0 and -2.5 is “low bone mass” (or “osteopenia”)
- T-score -2.5 or below is osteoporosis

However, there is an increasing trend to use risk factors with or without BMD to predict future fracture risk, for example the FRAX-score. When the risk is high enough, therapy is sometimes initiated even without performing a DXA to measure BMD [34, 35]. The

most common risk factors for osteoporotic fractures are: age, sex, immobility, previous fractures, familial fractures (especially a history of hip fractures of mothers), low body mass index, use of corticosteroids and smoking, low BMD and RA [34, 35].

Osteoporosis without any clear cause, apart from aging, is called primary osteoporosis. Several diseases and drugs may lead to an increased fragility of bone: i.e. rheumatoid arthritis, systemic lupus, ankylosing spondylitis, hyperthyroidism, hyperparathyroidism, celiac disease, corticosteroids and thyroid hormone, which is called secondary osteoporosis.

Bisphosphonates are the most frequently used drugs for treatment and prevention of osteoporosis. These drugs are synthetic analogues of pyrophosphate and inhibit osteoclast-mediated bone resorption. Bisphosphonates have demonstrated an increase of BMD and a decrease of vertebral and non-vertebral fracture risks in men and postmenopausal women [36, 37].

Rheumatoid arthritis and osteoporosis

Joint destruction is one of the hallmarks of rheumatoid arthritis. Joint destruction is a local loss of bones forming the joints, which can be divided into two types: peri-articular osteoporosis and joint erosions. Besides this local bone loss, there is also an increased generalized loss of bone in RA. Several studies have shown that patients with rheumatoid arthritis have a lower bone mass and a higher rate of bone loss than general population [38-41]. This low bone mass is associated with several disease characteristics such as disease duration, joint destruction, use of corticosteroids and disease activity (inflammation) [42-44], causing not only a decrease of bone mass but also a decrease of bone strength [45]. Consequently, there is also an increased rate of vertebral and non-vertebral fractures in patients with RA [46, 47].

In several studies there is a clear association between localized bone loss in RA and generalized loss of bone, indicating a common pathway for this loss of bone [48, 49]. There is not only this epidemiological evidence for an association between generalized and localized bone loss, but it also has a pathological substrate. In bone metabolism there is usually an equilibrium between bone formation (osteoblasts) and bone resorption (osteoclasts). This equilibrium is disturbed by the inflammation in RA.

Receptor Activator of NfKB (RANK) and osteopogesterin (OPG) system provide an aetiological explanation. RANK-ligand (RANKL) is an activator of osteoclast differentiation and activation, expressed on osteoblasts, fibroblasts and activated T-cells. OPG is the natural occurring decoy receptor for RANKL. Inflammatory cytokines, such as TNF and IL-1, involved in the inflammatory process of RA can induce expression of RANKL and thereby an increase of bone resorption [50, 51].

Another pathway involved in bone metabolism that is influenced by inflammation is the wingless (Wnt) signaling pathway. Wnt, a glycoprotein, is amongst others responsible for the differentiation of mesenchymal progenitors into osteoblasts. This process is inhibited by Dickkopf-1 (DKK-1) and sclerostin binding with the Wnt receptor complex on pre-osteoblasts, and it prohibits the binding of Wnt with its receptor [51, 52] (Figure 1). At least DKK-1 seems to be increased by TNF and it is associated with joint damage in RA patients [54]. Blocking DKK1 and sclerostin in mouse models of arthritis also prevents the formation of joint erosions and generalized bone loss [55, 56]. To conclude, there are mechanisms for inflammatory induced, increased bone resorption and decreased bone formation in RA.

OUTLINE OF THE THESIS

This thesis is divided into two parts. In the first part data are presented on our investigations into generalized bone loss and local bone loss in rheumatoid arthritis. In the second part data are shown of several investigations we undertook in our cohort of RA patients treated with infliximab (a TNF- blocker).

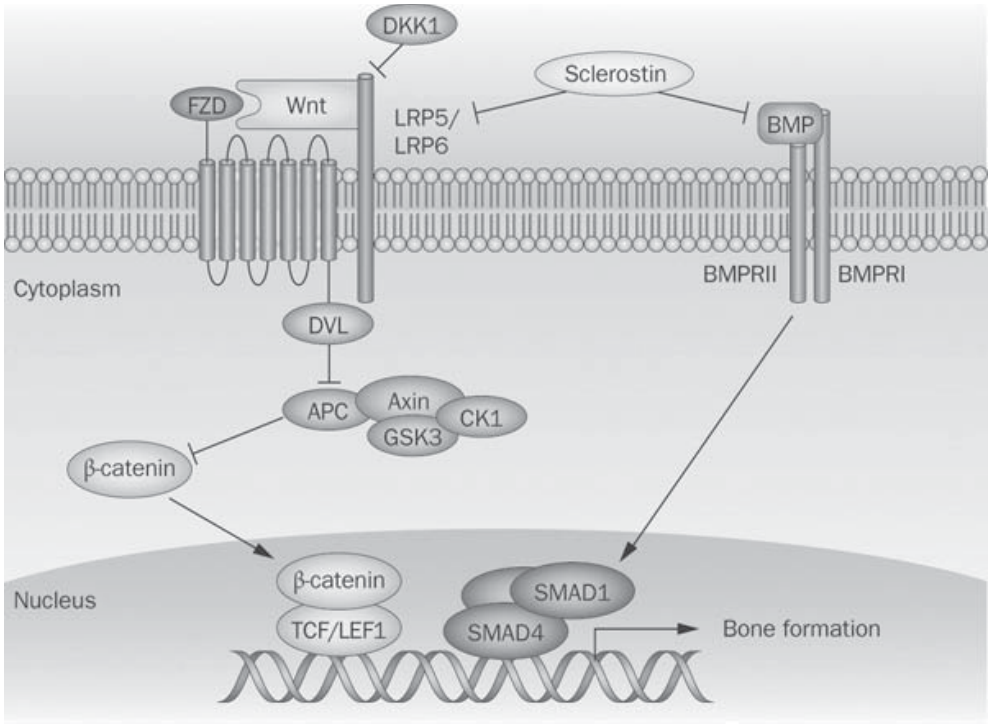

Figure 1 Wnt signalling pathway.[53]

Part 1

In **chapter 2 and 3** findings of our studies about the association between rheumatoid arthritis and osteoporosis are reported. In **chapter 2** short-term and long-term data on the effect of infliximab on markers of bone metabolism BMD in RA patients are shown. **Chapter 3** deals with data from the 5 year follow-up study of the OSTRA cohort, a cohort of female postmenopausal RA patients with an established disease. The OSTRA-group is an international collaboration between **OS**lo (Norway), **TR**uro (United Kingdom) and **A**msterdam (the Netherlands) studying bone (metabolism) in patients with inflammatory diseases. Data on fractures (vertebral and non-vertebral) and BMD of the spine, hip and hands are reported.

Part 2

In **chapter 4, 5, 6 and 7** data are shown about our cohort of RA patients treated with infliximab (anti-TNF), which we used for our study on several other aspects of rheumatoid arthritis and anti-TNF treatment. This cohort was established in Amsterdam in 2001: all RA patients treated with infliximab in the Jan van Breemen Institute, Slotervaartziekenhuis and VU University medical center were included and followed for several years or until termination of infliximab treatment.

There is mounting evidence of increased cardiovascular co-morbidity in RA. In **chapter 4** the effects are studied of inflammation and anti-TNF treatment on lipid levels. The effects of anti-TNF treatment on IgM-rheumatoid factor and anti-CCP levels are reported in **chapter 5**.

Treatment with infliximab is very effective in RA, although in several patients there is loss of efficacy after a number of infusions. **Chapter 6** shows the effects of the development of antibodies against infliximab on the clinical response to infliximab. The powerful immune suppression by anti-TNF raises concern about the side effects, especially regarding the risk of infections. In **chapter 7** data are presented about the occurrence of side effects in daily clinical practice in anti-TNF treated RA patients.

Quite a few patients with osteoporosis do not tolerate oral bisphosphonates because of gastro-intestinal complaints. In **chapter 8**, in an open randomized study, the effect of pamidronate intravenous infusions on BMD is compared with oral alendronate in patients with osteoporosis. Finally, a summary of the results and a conclusion is given in **chapter 9**.

REFERENCES

1 Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005; 4:130-6.

2 Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 1998; 41:778-99.

3 Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44-8.

4 Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002; 4(suppl 3):S265-S272.

5 Symmons DP. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. Best Pract Res Clin Rheumatol 2002; 16:707-22.

6 Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovský J, Wolfe F, Hawker G. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010; 62:2569-81.

7 Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50:380-6.

8 Chapuy-Regaud S, Sebbag M, Baeten D, Clavel C, Foulquier C, De Keyser F, Serre G. Fibrin deimination in synovial tissue is not specific for rheumatoid arthritis but commonly occurs during synovitides. J Immunol 2005; 174:5057-64.

9 Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. Arthritis Rheum 2004; 50:3485-94.

10 Kvien TK, Uhlig T. Quality of life in rheumatoid arthritis. Scand J Rheumatol 2005; 34:333-41.

11 Naz SM, Symmons DP. Mortality in established rheumatoid arthritis. Best Pract Res Clin Rheumatol 2007; 21:871-83.

12 Scott DL, Smith C, Kingsley G. Joint damage and disability in rheumatoid arthritis: an updated systematic review. Clin Exp Rheumatol 2003; 21(suppl 31):S20-S27.

13 Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM, Cathey MA. The mortality of rheumatoid arthritis. Arthritis Rheum 1994; 37:481-94.

14 Peters MJ, Symmons DP, McCarey D, Dijkmans BA, Nicola P, Kvien TK, McInnes IB, Haentzschel H, Gonzalez-Gay MA, Provan S, Semb A, Sidiropoulos P, Kitas G, Smulders YM, Soubrier M, Szekanecz Z, Sattar N, Nurmohamed MT. EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis. Ann Rheum Dis 2010; 69:325-31.

15 Peters MJ, Nielen MM, Raterman HG, Verheij RA, Schellevis FG, Nurmohamed MT. Increased cardiovascular disease in patients with inflammatory arthritis in primary care: a cross-sectional observation. J Rheumatol 2009; 36:1866-8.

16 Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov 2003; 2:473-88.

17 Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van Zeben D, Kerstens PJ, Hazes JM, Zwinderman AH, Peeters AJ, de Jonge-Bok JM, Mallée C, de Beus WM, de Sonnaville PB, Ewals JA, Breedveld FC, Dijkmans BA. Comparison of treatment strategies in early rheumatoid arthritis: a randomized trial. Ann Intern Med 2007; 146:406-15.

18 Quinn MA, Emery P. Window of opportunity in early rheumatoid arthritis: possibility of altering the disease process with early intervention. Clin Exp Rheumatol 2003; 21(suppl 31):S154-S157.

19 Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC, van Zeben D, Dijkmans BA, Peeters AJ, Jacobs P, van den Brink HR, Schouten HJ, van der Heijde DM, Boonen A, van der Linden S. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350:309-18.

20 Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; 350:2572-81.

21 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998; 41:1552-63.

22 Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 1999; 340:253-9.

23 Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA, Fischkoff SA, Chartash EK. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum 2003; 48:35-45.

24 Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999; 354:1932-9.

25 Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum 2002; 46:2287-93.

26 Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med 2004; 350:2167-79.

27 Aarden L, Ruuls SR, Wolbink G. Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement. Curr Opin Immunol 2008; 20:431-5.

28 Cummings SR, Black DM, Nevitt MC, Browner WS, Cauley JA, Genant HK, Mascioli SR, Scott JC, Seeley DG, Steiger P, et al. Appendicular bone density and age predict hip fracture in women. The Study of Osteoporotic Fractures Research Group. JAMA 1990; 263:665-8.

29 Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res 2002; 17:716-24.

30 van Staa TP, Dennison EM, Leufkens HG, Cooper C. Epidemiology of fractures in England and Wales. Bone 2001; 29:517-22.

31 Kanis JA, Johnell O, De Laet C, Johansson H, Oden A, Delmas P, Eisman J, Fujiwara S, Garnero P, Kroger H, McCloskey EV, Mellstrom D, Melton LJ, Pols H, Reeve J, Silman A, Tenenhouse A. A meta-analysis of previous fracture and subsequent fracture risk. Bone 2004; 35:375-82.

32 Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. Lancet 2002; 359:1761-7.

33 Kanis JA, Melton LJ, III, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994; 9:1137-41.

34 Kanis JA, Borgstrom F, De Laet C, Johansson H, Johnell O, Jonsson B, Oden A, Zethraeus N, Pfleger B, Khaltaev N. Assessment of fracture risk. Osteoporos Int 2005; 16:581-9.

35 Black DM, Steinbuch M, Palermo L, Dargent-Molina P, Lindsay R, Hoseyni MS, Johnell O. An assessment tool for predicting fracture risk in postmenopausal women. Osteoporos Int 2001; 12:519-28.

36 Liberman UA, Weiss SR, Bröll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. N Engl J Med 1995; 333:1437-43.

37 Harris ST, Watts NB, Genant HK, McKeever CD, Hangartner T, Keller M, Chesnut CH 3rd, Brown J, Eriksen EF, Hoseyni MS, Axelrod DW, Miller PD. Effects of risedronate treatment on vertebral and nonvertebral fractures in women with postmenopausal osteoporosis: a randomized controlled trial. Vertebral Efficacy With Risedronate Therapy (VERT) Study Group. JAMA 1999; 282:1344-52.

38 Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum 2000; 43:522-30.

39 Lems WF, Dijkmans BA. Should we look for osteoporosis in patients with rheumatoid arthritis? Ann Rheum Dis 1998; 57:325-7.

40 Haugeberg G, Ørstavik RE, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone loss in patients with rheumatoid arthritis: results from a population-based cohort of 366 patients followed up for two years. Arthritis Rheum 2002; 46:1720-8.

41 Magaro M, Tricerri A, Piane D, Zoli A, Serra F, Altomonte L, Mirone L. Generalized osteoporosis in non-steroid treated rheumatoid arthritis. Rheumatol Int 1991; 11:73-6.

42 Shibuya K, Hagino H, Morio Y, Teshima R. Cross-sectional and longitudinal study of osteoporosis in patients with rheumatoid arthritis. Clin Rheumatol 2002; 21:150-8.

43 Cortet B, Flipo RM, Blanckaert F, Duquesnoy B, Marchandise X, Delcambre B. Evaluation of bone mineral density in patients with rheumatoid arthritis. Influence of disease activity and glucocorticoid therapy. Rev Rhum Engl Ed 1997; 64:451-8.

44 Kvien TK, Haugeberg G, Uhlig T, Falch JA, Halse JI, Lems WF, Dijkmans BA, Woolf AD. Data driven attempt to create a clinical algorithm for identification of women with rheumatoid arthritis at high risk of osteoporosis. Ann Rheum Dis 2000; 59:805-11.

45 Lems WF. Bisphosphonates and glucocorticoids: effects on bone quality. Arthritis Rheum 2007; 56:3518-22.

46 van Staa TP, Geusens P, Bijlsma JW, Leufkens HG, Cooper C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54:3104-12.

47 Ørstavik RE, Haugeberg G, Mowinckel P, Høiseth A, Uhlig T, Falch JA, Halse JI, McCloskey E, Kvien TK. Vertebral deformities in rheumatoid arthritis: a comparison with population-based controls. Arch Intern Med 2004; 164:420-5.

48 Lodder MC, Haugeberg G, Lems WF, Uhlig T, Ørstavik RE, Kostense PJ, Dijkmans BA, Kvien TK, Woolf AD; Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Radiographic damage associated with low bone mineral density and vertebral deformities in rheumatoid arthritis: the Oslo-Truro-Amsterdam (OSTRA) collaborative study. Arthritis Rheum 2003; 49:209-15.

49 Tengstrand B, Hafstrom I. Bone mineral density in men with rheumatoid arthritis is associated with erosive disease and sulfasalazine treatment but not with sex hormones. J Rheumatol 2002; 29:2299-305.

50 Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423:337-42.

51 Walsh NC, Gravallese EM. Bone remodeling in rheumatic disease: a question of balance. Immunol Rev 2010; 233:301-12.

52 Rabelo Fde S, da Mota LM, Lima RA, Lima FA, Barra GB, de Carvalho JF, Amato AA. The Wnt signaling pathway and rheumatoid arthritis. Autoimmun Rev 2010; 9:207-10.

53 Choi, Y, Arron JR, Townsend MJ. Promising bone-related therapeutic targets for rheumatoid arthritis. Nat Rev Rheumatol 2009; 5:543-8.

54 Garnero P, Tabassi NC, Voorzanger-Rousselot N. Circulating dickkopf-1 and radiological progression in patients with early rheumatoid arthritis treated with etanercept. J Rheumatol 2008; 35:2313-5.

55 Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, Korb A, Smolen J, Hoffmann M, Scheinecker C, van der Heide D, Landewe R, Lacey D, Richards WG, Schett G. Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007; 13:156-63.

56 Eddleston A, Marenzana M, Moore AR, Stephens P, Muzylak M, Marshall D, Robinson MK. A short treatment with an antibody to sclerostin can inhibit bone loss in an ongoing model of colitis. J Bone Miner Res 2009; 24:1662-71.

Chapter 2a

Early changes in bone metabolism in patients with rheumatoid arthritis treated with infliximab

M. Vis
G.J. Wolbink
M.C. Lodder
R.J. van der Stadt
M.H.M.T. de Koning
P.J. Kostense
B.A.C. Dijkmans
W.F. Lems

Arthritis Rheum 2003 Oct; 48(10):2996-7

TO THE EDITOR

Rheumatoid arthritis (RA) is characterized by localized destruction of synovium, cartilage, and bone. Loss of bone in RA is not only localized in joints but is also generalized, and the latter (osteoporosis) is recognized as an extraarticular manifestation of the disease [1]. Bone loss in RA is related to disease activity, immobility and corticosteroid use [2]. Bone remodeling is a continuous process of bone resorption and bone formation. In RA, bone loss seems to be related to elevated bone resorption, while data on bone formation are conflicting [3-5]. Infliximab is effective in the treatment of RA: it causes marked reduction in disease activity and slows the radiologic progression of localized bone destruction [6]. Therefore, we undertook a study to determine its effect on markers of bone metabolism in patients with RA.

We studied consecutive patients with active RA, defined as a 28-joint Disease Activity Score (DAS-28) [7] of ≥3.2 who were treated with infliximab. Infliximab was administered by intravenous infusion at a dosage of 3 mg/kg at baseline and 2 weeks and 6 weeks thereafter. At each visit, data on disease activity (erythrocyte sedimentation rate, C-reactive protein level, disease activity rated on a visual analog scale, number of swollen and tender joints) and corticosteroid use were recorded. A patient was classified as a responder when the DAS-28 was decreased by at least 1.2 points at 6-week follow-up. Blood samples were collected on the morning of each infusion, and sera were stored immediately at -70 °C until assessment. Bone formation was measured by determining serum levels of osteocalcin (OC), N-propeptide of type I procollagen (PINP), and bone-specific alkaline phosphatase (BAP). Bone resorption was measured by determining serum levels of β-isomerized C-telopeptide of type I collagen (β-CTX) and C-propeptide of type I collagen (ICTP). All markers of bone metabolism were measured using commercial assays according to the instructions of the manufacturers (OC and β-CTX, measured with an Elecsys 2010 [Roche Diagnostics, Mannheim, Germany], BAP with an enzyme-linked immunosorbent assay [ELISA; Quidel, San Diego, CA], PINP and ICTP with a radioimmunoassay and an ELISA respectively [Orion Diagnostica, Helsinki, Finland]). Data on markers of bone metabolism were compared by paired t-test and Wilcoxon's signed rank test when appropriate.

Sixty-eight patients were included in the study: 54 women and 14 men, with a mean ± SD age of 55, ± 13.8 years and a median disease duration of 10 years (range <1 year–59 years). Forty-eight patients (71%) were IgM rheumatoid factor positive (>30 IU/ml). Sixty-one patients (90%) took methotrexate (median 15 mg/week), and 5 took other disease-modifying antirheumatic drugs during the study period. At baseline, 31 patients (46%) were taking corticosteroids (prednisone [median 10 mg/day]). In 19 of these patients the prednisone dosage remained stable during the study, whereas in 12 it was decreased. The mean ± SD DAS-28, 5.9 ± 1.4 at baseline, was decreased

significantly after infliximab treatment (4.6 ± 1.4 [p<0.001 versus baseline] at week 2 and 4.1 ± 1.5 [p<0.001 versus baseline] at week 6). Fifty-one patients (75%) were classified as responders and 17 as non-responders.

OC and PINP levels were significantly increased after 2 weeks and 6 weeks compared with baseline (p<0.001), while BAP levels did not change significantly (Table 1). When bone formation was analyzed only in the patients who took prednisone at a stable dosage or in those who did not take prednisone during the study, we also found a statistically significant increase in markers of bone formation. ICTP and β-CTX levels showed decreases, but only the decrease in the ICTP level at 6 weeks reached statistical significance (p<0.001) (Table 1). In the responder group, both markers of bone resorption (ICTP and β-CTX) were decreased significantly at 6 weeks compared with baseline (p<0.05). The change in levels of bone formation markers in the responder group was similar to that observed in the total group. In the nonresponder group, none of the markers of bone metabolism showed significant change. Changes in β-CTX levels were slightly correlated with changes in the DAS-28 (r=0.348, p=0.05), while changes in the other markers of bone metabolism were not significantly correlated with changes in disease activity.

Our data suggest that treatment with infliximab has a favorable effect on bone metabolism in patients with RA. Several studies have investigated levels of markers of bone metabolism in RA patients. Active RA was associated with increased levels of bone resorption in these studies [3, 4]. Our findings are in accordance with these data, since the decrease in disease activity of RA paralleled the decrease in markers of bone resorption. Reports on markers of bone formation are conflicting: both increased and decreased levels of bone formation markers in patients with RA have been reported

Table 1 Markers of bone metabolism in 68 rheumatoid arthritis patients at baseline and after 2 weeks and 6 weeks of infliximab treatment

	Baseline	Week 2	Week 6
Formation, mean ± SD			
OC, ng/ml	21.2 ± 11.4	23.0 ± 11.7†	23.9 ± 11.4†
PINP, µg/ml	43.9 ± 21.3	50.6 ± 23.7†	50.1 ± 21.1†
BAP, units/liter	22.3 ± 3.7	23.2 ± 4.8	22.7 ± 5.7
Resorption, median (interquartile range)			
ICTP, µg/ml	8.9 (7.1–12.3)	8.6 (6.8–11.6)	7.8 (6.2–10.2)†
β-CTX, ng/ml	0.32 (0.17–0.44)	0.29 (0.18–0.44)	0.29 (0.17–0.46)

OC, osteocalcin; PINP, N-propeptide of type I procollagen; BAP, bone-specific alkaline phosphatase; ICTP, C-propeptide of type I collagen; β-CTX, β-isomerized C-telopeptide of type I collagen.
† p<0.001 versus baseline.

[4, 5]. The observation that markers of bone formation, with the exception of BAP, increased during infliximab treatment was somewhat unexpected. Theoretically, the increase in bone formation could be the result of a decrease in the prednisone dosage in some patients [8]. However, in the present study this is unlikely: when we analyzed the patients who were not taking steroids and those who were taking steroids at a stable dosage during the study period, there were still significant increases in OC and PINP levels. Another possible explanation is that bone formation is depressed in active RA, as a result of disease activity. Thus, the increase in markers of bone formation might reflect the inhibition of suppressed bone formation in active RA. Whether long-term treatment with infliximab has a positive effect on bone metabolism, and subsequently on bone mineral density and fracture rate, will require further investigation.

REFERENCES

1 Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum 2000; 43:522-30.

2 Eggelmeijer F, Papapoulos SE, Westedt ML, van Paassen HC, Dijkmans BA, Breedveld FC. Bone metabolism in rheumatoid arthritis: relation to disease activity. Br J Rheumatol 1993; 32:387-91.

3 St Clair EW, Moak SA, Wilkinson WE, Sanders L, Lang T, Greenwald RA. A cross sectional analysis of 5 different markers of collagen degradation in rheumatoid arthritis. J Rheumatol 1998; 25:1472-9.

4 Cortet B, Flipo RM, Pigny P, Duquesnoy B, Boersma A, Marchandise X, Delcambre B. Is bone turnover a determinant of bone mass in rheumatoid arthritis? J Rheumatol 1998; 25:2339-44.

5 Hall GM, Spector TD, Delmas PD. Markers of bone metabolism in postmenopausal women with rheumatoid arthritis: effects of corticosteroids and hormone replacement therapy. Arthritis Rheum 1995; 38:902-6.

6 Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN; Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Infliximab and methotrexate in the treatment of rheumatoid arthritis. N Engl J Med 2000; 343:1594-602.

7 Prevoo MLL, van 't Hol MA, Kuper HH, van Leeuwen MA, van de Putte LBA, van Riel PLCM. Modified Disease Activity Scores that include twenty-eight–joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44-8.

8 Lems WF, Gerrits MI, Jacobs JWG. van Vugt RM, van Rijn HJ, Bijlsma JW. Changes in (markers of) bone metabolism during high dose corticosteroid pulse treatment in patients with rheumatoid arthritis. Ann Rheum Dis 1996; 55:288-94.

Chapter 2b

Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFκB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis

M. Vis
E.A. Haavardsholm
G. Haugeberg
T. Uhlig
A.E. Voskuyl
R.J. van der Stadt
B.A.C. Dijkmans
A.D. Woolf
T.K. Kvien
W.F. Lems

Ann Rheum Dis 2006 Nov; 65(11):1495-9

ABSTRACT

Objectives To examine whether treatment with anti-tumour necrosis factor (TNF) α prevents loss of bone mineral density (BMD) at the spine and hip (generalised) and in the hands (local) of patients with rheumatoid arthritis, and to study the changes in markers of bone metabolism, including receptor activator of the NFκB ligand (RANKL) and osteoprotegerin (OPG), during anti-TNF treatment.

Patients and methods: In total 102 patients with active rheumatoid arthritis, who were treated with infliximab during 1 year, were included in this open cohort study. The BMD of the spine and hip (dual X-ray absorptiometry) and hands dual X-ray radiogrammetry was measured before the start of treatment and after 1 year. Changes in osteocalcin formation, β-isomerised carboxy terminal telopeptide of type 1 collagen (β-CTx, resorption), RANKL and OPG were determined at 0, 14, 30 and 46 weeks.

Results: The BMD of the spine and hip was unchanged during treatment with infliximab, whereas BMD of the hand decreased significantly by 0.8% ($p<0.01$). The BMD of the hip in patients with a good European League Against Rheumatism response showed a favourable change compared with patients not achieving such a response. Serum β-CTx and RANKL were both considerably decreased compared with baseline at all time points. The decrease in β-Tx was associated with the decrease in Disease Activity Score of 28 joints and C reactive protein during the 0–14 weeks interval.

Conclusion: In patients with rheumatoid arthritis treated with infliximab, spine and hip bone loss is arrested, whereas metacarpal cortical hand bone loss is not stopped. The outcome of the study also supports a relationship between clinical response, in terms of reduced inflammatory activity, and changes in bone loss of the spine, hip and hands.

INTRODUCTION

Bone loss in the joints is one of the hallmarks of rheumatoid arthritis. This local bone loss can be divided into erosions of the joint and periarticular osteoporosis. Apart from this localised process, generalised bone loss (osteoporosis) is increased in patients with rheumatoid arthritis [1-3]. Recent literature suggests that these two types of bone loss are at least partly mediated through the same mechanism. A previous study from the Oslo-Truro-Amsterdam group showed that the Larsen Score for joint damage was associated with bone mineral density (BMD) of the hip and the occurrence of vertebral deformities in patients with rheumatoid arthritis [4, 5]. Other clinical studies in patients with rheumatoid arthritis found similar associations between osteoporosis and radiographic joint damage [6-10]. The receptor activator of the NFκB ligand (RANKL) and osteoprotegerin (OPG) system provides a theoretical background for such a common mechanism. RANKL is a membrane-bound protein and a key factor in the activation and differentiation of osteoclasts by binding to its receptor RANK on premature osteoclasts. OPG is the antagonist of RANKL and suppresses the differentiation and activation of osteoclasts [11, 12]. RANKL expression is one among the factors up regulated by several proinflammatory cytokines — that is, tumour necrosis factor (TNF)α and interleukin 1 (IL1) [13, 14]. Currently, the most effective drugs for the treatment of rheumatoid arthritis are the TNFα blocking agents (infliximab, etanercept and adalimumab). Randomised controlled trials have shown that these drugs retard radiographic joint destruction [15, 16]. This finding raises the question whether these agents also have the ability to prevent generalised bone loss in the spine and hip, and local bone loss in the hands in patients with rheumatoid arthritis. We aimed to address this question and to study changes in markers of bone metabolism, including RANKL and OPG, during such treatment.

PATIENTS AND METHODS

Patients

Consecutive patients with rheumatoid arthritis from four rheumatology departments, VU University Medical Centre (VUMC), Jan van Breemen Institute (JBI), Slotervaart Hospital, Amsterdam, the Netherlands, and Diakonhjemmet Hospital, Oslo, Norway, who were treated with infliximab for at least 1 year and had dual X-ray absorptiometry (DEXA) measurements at baseline and at follow-up were included. All patients fulfilled the American College of Rheumatology criteria for rheumatoid arthritis, had active disease (Disease Activity Score of 28 joints (DAS-28) 3.2), and had previously failed at least two disease modifying antirheumatic drugs (DMARDs), including methotrexate

[17, 18]. In total, 138 patients were included in the cohort, but 36 patients dropped out in the first year of treatment: 28 because of non-response (78%), 6 because of side effects (16%) and 2 patients (6%) who died owing to infliximab-unrelated events. Thus, the data from 102 patients were eligible for analyses (Table 1). The baseline characteristics of the dropout patients were comparable to those of the patients included in the study (data not shown).

Methods

Infusions with infliximab were given at 0, 2, 6 and 14 weeks, and after that at an interval of 8 weeks. Infliximab was given intravenously at a starting dose of 3 mg/kg. The dose of infliximab could be increased to 7.5 mg/kg in patients who showed inadequate response, according to clinical judgement. A dose increment occurred in 21 patients.

Demographics and disease-related data

The demographic data collected at baseline were recorded from medical history and patients' medical records. The following variables were collected: age, sex, disease

Table 1 Baseline characteristics of the patients with rheumatoid arthritis (N=102)

Demography	
Age (years), mean (SD)	53 (13)
Sex, female, n (%)	84 (82)
Disease duration years, median (range)	8 (1–49)
Erosive disease, n (%)	84 (82)
IgM-RF positive, n (%)	75 (74)
Disease activity	
DAS-28, mean (SD)	5.4 (1.2)
ESR (mm/h), mean (SD)	32 (23)
CRP (mg/l), median (range)	14 (1–174)
Drugs	
Methotrexate, n (%)	98 (96)
Prednisone, n (%)	46 (45)
Anti-osteoporotic drugs, n (%)	26 (26)
BMD	
Vertebral spine (g/cm^2), mean (SD), n=102	0.993 (0.19)
t-score, mean (SD)	-0.82 (1.6)
z-score, mean (SD)	-0.09 (1.5)
Total hip (g/cm^2), mean (SD), n=89	0.830 (0.14)
t-score, mean (SD)	-1.02 (1.2)
z-score, mean (SD)	-0.51 (1.2)
Hand, mean (SD), n=53	0.497 (0.98)

duration, presence or absence of bony erosions, serum rheumatoid factor status (positive if immunoglobulin-rheumatoid factor >30 U/l), current and previous use of DMARDs, anti-osteoporotic drugs and corticosteroids.

Disease activity

The patients were assessed at each visit with core measures of disease activity, and the DAS-28 score was computed. The European League Against Rheumatism (EULAR) response criteria were applied to define response [17].

BMD of hip and spine

BMD was measured before and after 1 year of infliximab treatment using (DEXA). A total of 102 patients had a DEXA measurement of the spine, and in 89 patients DEXA measurements of the hip were available. The DEXA machines used were Hologic 4500 (Hologic, Waltham, Massachusetts, USA) in the Slotervaart hospital and the VUMC, a Lunar Expert (Lunar, Madison, Wisconsin, USA) at the Diakonhjemmet Hospital, and a Lunar DPX (Lunar) at the JBI. The same DEXA machine used at baseline was used for follow-up measurement for each patient. The coefficients of variation, measured with a local spine phantom for the different centres, were acceptable and comparable: the VUMC 0.56%, Slotervaart Hospital 0.48%, JBI 0.4% and Diakonhjemmet Hospital 0.8%. The BMD data of spine and hip (g/cm^2) and t- and z-scores were determined using the local reference population provided by the manufacturer of the DEXA machine.

BMD of the hand

BMDs of the hand at baseline and after 1 year were measured on plain radiographs of the left and right hand (anteroposterior view) using digital X-ray radiogrammetry (DXR; Pronosco X posure system, system 2.0, Sectra, Linkaping, Sweden). This method measures cortical thickness from regions of interest at the centre of the second, third and fourth metacarpals, and a mean BMD surrogate is calculated. The theoretical background for this method has been fully described [19, 20]. The coefficient of variation for this method was 0.25% [21]. The Pronosco DXR system we used is validated only for evaluation of conventional radiographs. Fifty three patients had conventional radiographs available for evaluation. The baseline characteristics and change in disease activity of the subgroup of patients with hand radiographs were comparable to those of the whole group. The patient data for BMD of the hand (g/cm^2) are given as the mean of the right and left hand.

Markers of bone metabolism

In a total of 72 patients serum samples were available for evaluation. These patients also had baseline characteristics and change in disease activity similar to that of the entire patient group. Serum was collected in the morning (non-fasting) before each infusion and stored immediately at -20 °C or lower until analyses. Markers of bone metabolism were measured at 0, 14, 28 and 46 weeks. Bone formation was measured by osteocalcin, and bone resorption was determined by β-isomerised carboxy terminal telopeptide of type 1 collagen (β-CTx) using commercial assays according to the instructions of the manufacturer (Roche Diagnostics, Elecsys 2010, Mannheim, Germany). Levels of osteoclast regulating proteins, including the soluble RANKL and OPG, were determined in serum using an ELISA from Immun diagnostik (Bensheim, Germany). All assays had an intra-assay and interassay coefficient of variation of <5%.

Statistical analysis

Paired t-tests and Wilcoxon rank tests were used to examine longitudinal changes in BMD and markers of bone metabolism, where appropriate. Subgroup analyses were carried out to investigate differences in change in BMD of the spine, the hip and the hand in different groups. Patients were categorised according to achieved EULAR response, use of prednisolone and bisphosphonate, and presence of radiographic erosions. Changes in BMD between these subgroups were analysed using an independent Student's t-test or Mann-Whitney U test as necessary.

Associations between changes in BMD and changes in disease activity and bone markers were also examined in a multivariate linear regression model adjusted for age, sex, disease duration, erosive disease, prednisolone and bisphosphonate use.

Similar regression analyses were carried out with bone markers as the dependent variable and disease activity as independent variable. The changes in markers of bone metabolism and the association with disease activity markers were investigated separately between the different time points: 0–14, 0–30 and 0–46 weeks.

All data were analysed using the SPSS V.11.1 software package. A value of $p<0.05$ was considered to be significant.

RESULTS

The mean (standard deviation (SD)) disease activity measured by DAS-28 decreased from 5.5 (1.2) at baseline to 3.8 (1.4) after 14 weeks, 3.6 (1.3) after 30 weeks and 3.5 (1.4) after 46 weeks ($p<0.001$ at all time points compared with baseline). In all, 40

patients had a good response, 43 patients a moderate response and 19 patients a non-response at 46 weeks as defined by the EULAR response criteria.

Bone mineral density

One fifth of the 102 patients had osteoporosis (t-score <-2.5 at either spine or hip), and osteopenia (t-score <-1.5 but >-2.5 at either spine or hip) was present in almost half (47%) of the patients. A considerably lower BMD of the spine and hip was observed at baseline in patients with erosive disease than in patients with non-erosive disease (data not shown).

BMD of the vertebral spine and hip remained unchanged during treatment with infliximab. In contrast, BMD of the hand decreased significantly by 0.004 g/cm^2 (-0.8%) during the year ($p<0.05$; Table 2). The mean z scores of the spine and hip showed an increasing trend: 0.039 and 0.023, respectively ($p=0.334$ and 0.376).

Using the smallest detectable difference for each measurement, we calculated the number of patients who lost, gained and maintained BMD for each site (Table 3).

There was no significant difference in change in BMD between the patients from different centres, patients with and without bone markers for evaluation, and patients with and without DXR measurements.

Table 2 Mean percentage change (SD %) in the bone mineral density of the spine, hip and hand for all patients and for patients with (40%) and without (60%) good European League Against Rheumatism (EULAR) response

		Good EULAR response	
	All	No	Yes
Vertebral-spine (n=102)	0.20 (5.1)	-0.57 (5.0)	0.74 (5.3)
Total hip (n=89)	-0.20 (3.6)	-0.68 (3.8)	0.77 (3.4)*
Hand (n=53)	-0.82 (2.4)	-1.2 (2.6)	-0.63 (2.3)

* $p<0.001$ versus those without good response.

Table 3 Number of patients losing, maintaining and gaining bone mineral density during 1-year treatment with infliximab

	Gain n (%)	Maintain, n (%)	Lose, n (%)
Vertebral-spine (n=102)	32 (31)	39 (38)	31 (30)
Total hip (n=89)	14 (16)	61 (68)	15 (16)
Hand (n=53)	6 (11)	22 (42)	25 (47)

Markers of bone metabolism

The bone resorption marker, β-CTx (ng/ml), decreased considerably from 0 to 14 weeks and remained markedly decreased during the rest of the year (Figure 1). The marker for bone formation, osteocalcin (ng/ml), was appreciably increased at 14 weeks compared with baseline, but not at 30 and 46 weeks (Figure 1B). RANKL (pmol/ml) decreased markedly during the year whereas OPG (pmol/ml) did not change markedly. This resulted in a favourable change in the RANKL/OPG ratio.

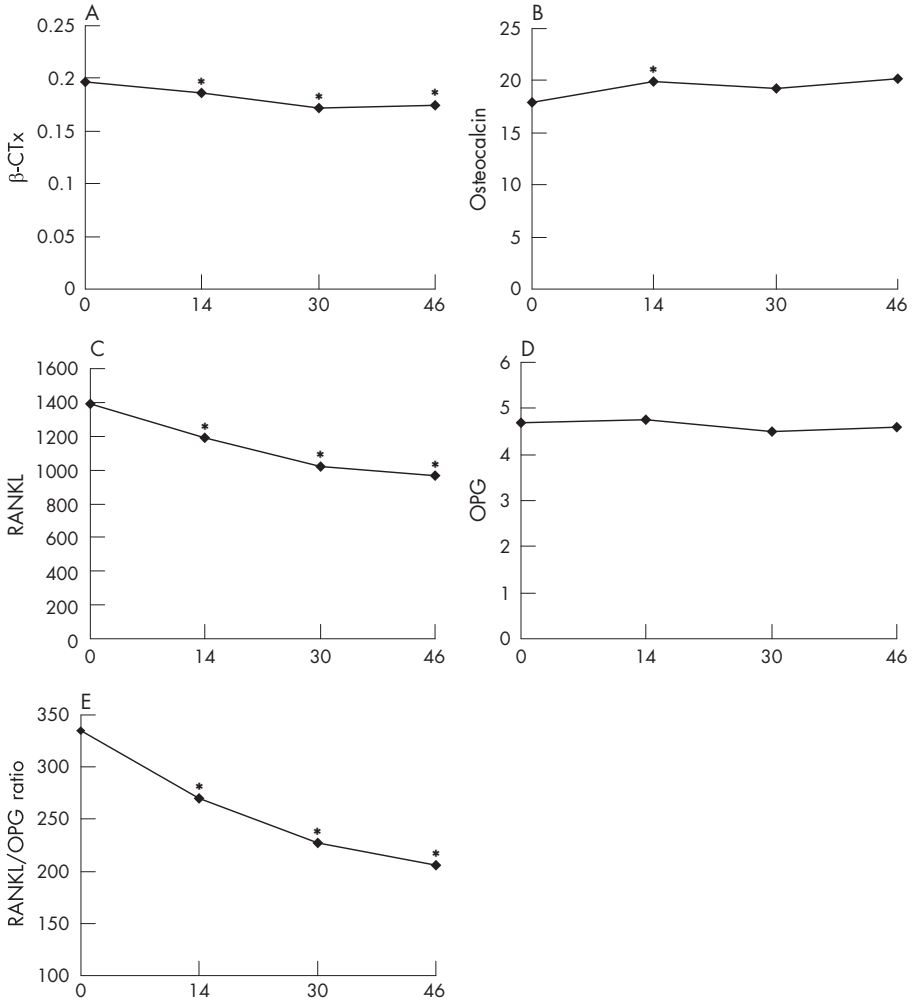

Figure 1 (A–E) Changes in median β-isomerised carboxy terminal telopeptide of type 1 collagen (β-CTx, resorption), osteocalcin formation, receptor activator of the NFκB ligand (RANKL), osteoprotegerin (OPG) and RANKL/OPG ratio during treatment with infliximab. * $p<0.05$ versus baseline.

Associations between changes in disease activity, BMD and bone markers

The change in the BMD of the spine, hip and hand was numerically larger in patients without good EULAR response than in patients achieving a good response (a decrease in DAS>1.2 resulting in a DAS<3.2; Table 2). This difference was significant between these two groups at the hip.

The BMD changed non-significantly in both the prednisone users and the non prednisone users, by +0.2% and -0.04% at the spine, by -0.2% and -0.28% at the hip, and by -0.9% and -0.7% at the hand, respectively. No significant difference was found in change in BMD between the prednisone and non prednisone users.

Subgroup analysis for bisphosphonate use also did not show any difference in the change in BMD between the users and non-users.

In the multivariate linear regression model investigating the influence of disease activity on markers of bone metabolism, we found a relationship between the change in β-CTx and the change in DAS-28 and C reactive protein during the 0–14 week time interval ($C=0.302$, $B=0.015$, $p<0.05$ and $C=0.315$, $B=0.002$, $p<0.05$, respectively). At the other time intervals there was no significant relationship between β-CTx and markers of disease activity. OPG showed a weak inverse association with the change in DAS-28 at all the time intervals (data not shown). No significant association was found between changes in disease activity and levels of osteocalcin or RANKL at any of the time intervals.

DISCUSSION

The main findings of this study were that 1 year treatment with infliximab arrests generalised bone loss in patients with rheumatoid arthritis, without stopping the localised bone loss at the hands. An increased generalised bone loss is usually found in observational studies of patients with rheumatoid arthritis owing to the negative effects of rheumatoid arthritis on bone loss (disease activity and immobilisation) [2, 3]. We compared our results with those of historical control groups from earlier studies of patients with established rheumatoid arthritis, which have shown a bone loss of up to 0.7% at the spine and -1.1% at the hip during 1 year, when treated with conventional DMARDs [22-24]. The generalised annual bone loss in patients with early rheumatoid arthritis is even larger: -4.2% at the spine and -2.1% at the hip [25, 26]. Our study population consisted of patients with early and established rheumatoid arthritis, and 27 patients had disease duration <3 years, emphasising the favourable changes in BMD we found in our study. The z-scores in our patients showed an increasing

trend, supporting the observation that treatment with infliximab arrests loss of BMD in patients with rheumatoid arthritis. Comparison of our data with the data derived from historical control groups having methodological limitations clearly shows that bone loss is far less in patients treated with an active regimen of TNFα blockers. In contrast with the effects on BMD of the spine and the hip, BMD of the hand measured by DXR decreased considerably. There is a lack of data on hand BMD measured by DXR in patients with established rheumatoid arthritis treated with conventional DMARDs. Therefore we do not know how this bone loss at the hand compares with other cohorts of patients with rheumatoid arthritis. A comparable finding was observed in a small pilot study comparing patients with rheumatoid arthritis (n=10 in each group) treated with infliximab or methotrexate. In this study, the bone loss was fully arrested in the infliximab group at the spine and hip but not at the hands [27]. In the present study, patients with a good EULAR response had increased BMD of the spine and hip. However, bone loss at the hand was also not fully arrested in the good responder group. The bone loss at the hands may suggest that the negative effects of patients with rheumatoid arthritis are not fully blocked. This hypothesis of a suboptimal anti-inflammatory control at the hands is supported by findings from a recently published double-blind randomised controlled trial showing that the rate of hand bone loss measured by DXR was considerably lower in patients with rheumatoid arthritis treated with prednisolone than in patients treated with placebo, but dropped markedly over time in both treatment groups [21]. In the large randomised controlled trial of patients with rheumatoid arthritis treated with infliximab, radiological damage was also not fully arrested, especially in patients treated with infliximab 3 mg/kg. Radiological joint damage was, however, fully arrested in the high dose infliximab group (10 mg/kg) [16, 28]. Thus, despite infliximab having a favourable effect on the disease activity in most patients, it might not fully block the radiological damage and bone loss at the hands at a dose of 3 mg/kg. Bone resorption, as depicted by β-CTx, decreased during the treatment with infliximab (as expected), whereas bone formation (assessed by osteocalcin) increased. We found that the change in disease activity (DAS-28 and C reactive protein) between 0 and 14 weeks was independently related to the change in bone resorption marker β-CTx between 0 and 14 weeks. This supports the view that infliximab reduces disease activity and thereby prevents bone loss. The change in osteocalcin was not observed in patients not using prednisone, and was more pronounced in the patients with rheumatoid arthritis using prednisone (data not shown). Prednisone is a well-known suppressor of bone formation [29]. Hence, the increase in osteocalcin is probably induced by a decrease in prednisone dosage during the study, because of the good clinical response to infliximab. The osteoclast activating protein RANKL showed a linear decrease during the treatment with infliximab. This resulted in a favourable change in the RANKL/OPG ratio. In the

Combinatie Therapie Bij Reumatoïde Artritis study (COBRA), an intervention study in patients with early rheumatoid arthritis, the RANKL/OPG ratio at baseline was a good predictor of radiological joint damage [30]. Overall, the changes in bone markers during 1 year with infliximab treatment support the bonesparing effect of infliximab observed in our study. In summary, our study shows that in patients with rheumatoid arthritis treated with infliximab, loss of BMD at the hip and spine is arrested, but not that at the hands. The fact that bone loss was arrested at the hip and the spine seems to be to a large extent due to a decrease in disease activity.

REFERENCES

1 Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum 2000; 43:522-30.

2 Lane NE, Pressman AR, Star VL, Cummings SR, Nevitt MC. Rheumatoid arthritis and bone mineral density in elderly women. The Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1995; 10:257-63.

3 Lems WF, Dijkmans BA. Should we look for osteoporosis in patients with rheumatoid arthritis? Ann Rheum Dis 1998; 57:325-7.

4 Haugeberg G, Lodder MC, Lems WF, Uhlig T, Ørstavik RE, Dijkmans BA, Kvien TK, Woolf AD. Hand cortical bone mass and its associations with radiographic joint damage and fractures in 50–70 year old female patients with rheumatoid arthritis: cross sectional Oslo Truro Amsterdam (OSTRA) collaborative study. Ann Rheum Dis 2004; 63:1331-4.

5 Lodder MC, Haugeberg G, Lems WF, Uhlig T, Orstavik RE, Kostense PJ, Dijkmans BA, Kvien TK, Woolf AD; Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Radiographic damage associated with low bone mineral density and vertebral deformities in rheumatoid arthritis: the Oslo Truro Amsterdam (OSTRA) collaborative study. Arthritis Rheum 2003; 49:209-15.

6 Berglin E, Lorentzon R, Nordmark L, Nilsson-Sojka B, Rantapää Dahlqvist S. Predictors of radiological progression and changes in hand bone density in early rheumatoid arthritis. Rheumatology (Oxford) 2003; 42:268-75.

7 Forsblad D'Elia H, Larsen A, Waltbrand E, Kvist G, Mellström D, Saxne T, Ohlsson C, Nordborg E, Carlsten H. Radiographic joint destruction in postmenopausal rheumatoid arthritis is strongly associated with generalised osteoporosis. Ann Rheum Dis 2003; 62:617-23.

8 Forslind K, Keller C, Svensson B, Hafström I; BARFOT Study Group. Reduced bone mineral density in early rheumatoid arthritis is associated with radiological joint damage at baseline and after 2 years in women. J Rheumatol 2003; 30:2590-6.

9 Jensen T, Hansen M, Jensen KE, Pødenphant J, Hansen TM, Hyldstrup L. Comparison of dual X-ray absorptiometry (DXA), digital X-ray radiogrammetry (DXR), and conventional radiographs in the evaluation of osteoporosis and bone erosions in patients with rheumatoid arthritis. Scand J Rheumatol 2005; 34:27-33.

10 Stewart A, Mackenzie LM, Black AJ, Reid DM. Predicting erosive disease in rheumatoid arthritis. A longitudinal study of changes in bone density using digital X-ray radiogrammetry: a pilot study, Rheumatology (Oxford) 2004; 43:1561-4.

11 Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423:337-42.

12 Hofbauer LC, Kuhne CA, Viereck V. The OPG/RANKL/RANK system in metabolic bone diseases. J Musculoskelet Neuronal Interact 2004; 4:268-75.

13 Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin 6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 1999; 25:255-9.

14 Nakashima T, Kobayashi Y, Yamasaki S, Kawakami A, Eguchi K, Sasaki H, Sakai H. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 2000; 275:768-75.

15 Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, Fischkoff SA, Chartash EK. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo controlled, 52-week trial. Arthritis Rheum 2004; 50:1400-11.

16 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998; 41:1552-63.

17 van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39:34-40.

18 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

19 Rosholm A, Hyldstrup L, Backsgaard L, Grunkin M, Thodberg HH. Estimation of bone mineral density by digital X-ray radiogrammetry: theoretical background and clinical testing. Osteoporos Int 2001; 12:961-9.

20 Jørgensen JT, Andersen PB, Rosholm A, Bjarnason NH. Digital X-ray radiogrammetry: a new appendicular bone densitometric method with high precision. Clin Physiol 2000; 20:330-5.

21 Haugeberg G, Strand A, Kvien TK, Kirwan JR. Reduced loss of hand bone density with prednisolone in early rheumatoid arthritis results from a randomized placebo controlled trial. Arch Intern Med 2005; 165:1293-7.

22 Dolan AL, Moniz C, Abraha H, Pitt P. Does active treatment of rheumatoid arthritis limit disease-associated bone loss? Rheumatology (Oxford) 2002; 41:1047-51.

23 Haugeberg G, Ørstavik RE, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone loss in patients with rheumatoid arthritis: results from a population-ased cohort of 366 patients followed up for two years. Arthritis Rheum 2002; 46:1720-8.

24 Shibuya K, Hagino H, Morio Y, Teshima R. Cross-sectional and longitudinal study of osteoporosis in patients with rheumatoid arthritis. Clin Rheumatol 2002; 21:150-8.

25 Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC, van Zeben D, Dijkmans BA, Peeters AJ, Jacobs P, van den Brink HR, Schouten HJ, van der Heijde DM, Boonen A, van der Linden S. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350:309-18.

26 Gough AK, Lilley J, Eyre S, Holder RL, Emery P. Generalised bone loss in patients with early rheumatoid arthritis. Lancet 1994; 344:23-7.

27 Quinn M, Conaghan P, Haugeberg G, Greenstein A, Emery P. The effect of TNF blockade on bone loss in early RA. Arthritis Rheum 2002; 46(suppl):S519.

28 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, St Clair EW, Keenan GF, van der Heijde D, Marsters PA, Lipsky PE; Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004; 50:1051-65.

29 Lems WF, Gerrits MI, Jacobs JW, van Vugt RM, van Rijn HJ, Bijlsma JW. Changes in (markers of) bone metabolism during high dose corticosteroid pulse treatment in patients with rheumatoid arthritis. Ann Rheum Dis 1996; 55:288-93.

30. van Tuyl LH, Voskuyl AE, Boers M, Geusens P, Landewé RB, Dijkmans BA, Lems WF. Baseline RANKL:OPG ratio and markers of bone and cartilage degradation predict annual radiological progression over 11 years in rheumatoid arthritis. Ann Rheum Dis 2010; 69:1623-8.

Chapter 3a

High incidence of vertebral and non-vertebral fractures in the OSTRA cohort study: a 5 year follow-up study in postmenopausal women with rheumatoid arthritis

M. Vis
E.A. Haavardsholm
P. Bøyesen
G. Haugeberg
T. Uhlig
M. Hoff
A. Woolf
B. Dijkmans
W. Lems
T.K. Kvien

Osteoporos Int 2011 Jan 13 (Epub ahead of print)

ABSTRACT

Introduction: To investigate the incidence of vertebral and non-vertebral fractures over a 5-year period in a cohort of postmenopausal patients with established rheumatoid arthritis (RA).

Methods: 150 female patients with established RA were included into the OSTRA cohort. The cohort was assessed at baseline and at 5 years for incident vertebral and non-vertebral fractures. Spinal X-rays were taken at baseline and at follow-up and scored using the semi-quantitative method according to Genant.

Results: At 5 years, 102 patients (68%) were examined and included in the present analysis. At baseline, the mean age was 61 years, disease duration 17 years, body mass index 25.5 kg/m^2 and 65% of the patients were rheumatoid factor positive. Fifteen percent were treated with bisphosphonates, 25% received calcium supplementation and 20% vitamin-D supplementation at baseline. During 5-year follow-up a total of 16 patients out of 102 patients (16%) had a new non-vertebral fracture (annual incidence of 3.2 (95% CI 1.8–5.5) per 100 patients/year). In 18 patients out of 97 patients (19%), new vertebral fractures were identified on spinal X-ray (annual incidence of 3.7 (95% CI 2.2–5.8) per 100 patients/year).

Conclusions: We found a high incidence of vertebral and non-vertebral fractures in a cohort of women with established RA compared to population based studies.

INTRODUCTION

Osteoporosis is a well known extra-articular feature of rheumatoid arthritis (RA). Bone mineral density (BMD) is decreased in patients with RA [1, 2]. The clinical endpoint of osteoporosis, fractures are also more prevalent in RA patients compared to the general population [3-5]. Reasons for this decreased BMD and increased prevalence of fractures in RA include among others inflammation, reduced physical activity and corticosteroid use [2]. Almost all data regarding osteoporosis in RA is generated from cross sectional studies. Longitudinal studies are scarce, especially studies with a focus on fractures. Recently, van Staa et al. reported, that in a large case-control study the risk of fractures was about 1.5 times higher in RA patients than in healthy controls [4]. In this study only clinical fractures were assessed and spinal X-rays were not performed routinely to identify asymptomatic vertebral fractures. However these asymptomatic fractures are also associated with an increased risk of new fractures and with an increased morbidity [6, 7].

The OSTRA-group (OSlo, TRuro, Amsterdam) is an international collaboration investigating osteoporosis in RA. Five years ago the OSTRA-group performed a study in postmenopausal patients with RA and found that radiological joint damage (total Larsen-score) was associated with a low BMD and vertebral fractures [8].

To further clarify the association between RA and osteoporosis we performed a 5-year follow-up assessment of this cohort. The main objective of this 5-year follow-up study was to evaluate the incidence of vertebral and non-vertebral fractures in female post-menopausal RA patients.

PATIENTS AND METHODS

Patients

All 150 patients from the original study were eligible to participate in the follow-up study. The inclusion and exclusion criteria for the baseline study have previously been described in detail [8]. In short, in each of 3 centers, general rheumatology clinics in Oslo (Norway), Truro (UK), and Amsterdam (the Netherlands), 50 female patients were consecutively enrolled. The patients included were 50–70 years old and fulfilled the American College of Rheumatology (formerly American Rheumatism Association) 1987 revised classification criteria for RA. The disease duration of all patients was 5 or more years [9]. In total, 102 patients of the original cohort consented to a follow-up assessment (33 from Oslo, 34 from Truro, and 35 from Amsterdam). The main reasons for not participating in the follow-up study were: 15 moved away from the hospital

area, 5 suffered from severe co-morbidity, 8 had died and 20 did not participate for unknown reasons or could not be contacted. The baseline characteristics of the 102 patients, who had a follow-up measurement did not differ form the characteristics of all the 150 patients at baseline and of those patients (n=42) that dropped out ((lowest p=0.282) data not shown).

Demographics and medical history

Data at follow-up were collected from interviews, clinical examination, questionnaires and patient's medical records and included:, height, weight, calcium intake, history of falls (number of falls during the last year and cause) and fractures (anatomical site and cause), current and previous use of anti-osteoporotic (anti resorptive therapy (ART) and hormone replacement therapy (HRT)) and disease-modifying anti-rheumatic drugs (DMARDs) and history of corticosteroid use (previous and current use, cumulative amount over the past 5 years, use of ≥7.5 mg for >6 months, and number of months on corticosteroids). Physical disability was assessed by means of the Health Assessment Questionnaire (HAQ; 20 items, score range 0–3, with higher scores indicating worse disability) [10].

Disease activity

Measures of RA disease activity were assessed with visual analogue scales (0–100 mm) of pain and patient's global disease activity; 28 tender and swollen joint counts, and acute phase reactants (the erythrocyte sedimentation rate [ESR; mm/hour] and C-reactive protein [CRP; mg/L], both measured with standardized local measurement techniques). The modified 28 joints disease activity score (DAS-28) was calculated according to published guidelines [11]. Joint scores were performed by experienced rheumatology nurses in Oslo and Truro and in Amsterdam by a physician (MV). The mean ESR and CRP were calculated based on all available measurements during the 5-year follow-up.

Fractures

Non-vertebral

Non-vertebral fractures and their cause were assessed at 5-year follow-up by interview and were validated by checking available data (radiology reports and chart review). Low-energy traumatic fractures (i.e. a fall from standing height) were regarded as osteoporotic fractures.

Vertebral

All spinal X-rays were taken according to local protocol, the same protocol was used at baseline and follow-up. Lateral radiographs of the spine were scored according to the semi-quantitative method described by Genant et al. [12]. Scoring was performed individually by 2 trained observers (MV and WL) and consensus both at baseline and follow-up was obtained in cases of discrepancies between both observers. Follow-up radiographs were scored blinded for the baseline image, and the results were subsequently compared to the baseline X-rays and scores to see if new vertebral fractures were detected. A fracture was scored as an incident vertebral fracture if it was not present at baseline or if there was a significant increase in loss of height (more than 20%) in a vertebra which was already fractures at baseline.

Ethics

The study protocol was approved by the local medical ethical committees of the 3 centers and all patients gave written informed consent.

Statistical analysis

Patients with incident fractures (vertebral or non-vertebral fractures) were compared to those not having a new fracture with regard to demographic variables, clinical variables and BMD using 2-sided t-tests for continuous variables and chi-square tests for counts. The incidence of patients with fractures was expressed per 100 patients/year with 95% confidence intervals (CI). Possible predictors of incident vertebral and non-vertebral fractures were subsequently examined in a multivariate logistic regression analysis. The criteria for entering independent variables in the logistic regression analysis was a $p<0.2$ in the univariate analysis and a supposed clinical relevance for the dependent variable. We were able to build a prediction model with only significant covariates by using backward stepwise elimination of the least significant covariate. All statistical analyses were performed using the SPSS (Chicago, IL), version 15.0.

RESULTS

Patient characteristics

The clinical characteristics of the 102 patients included in this study are presented in Table 1. At baseline the patients had a mean (SD) age of 61 (6) years with a median (range) disease duration of 17 (6–25) years, 83% of the patients had erosive disease and 65% patients were rheumatoid factor positive.

Table 1 Characteristics of the 102 patients with RA included in the 5-year follow-up

		Baseline	Follow-up
Age [years]	Mean (SD)	61 (6)	na
Disease duration [years]	Median (range)	17 (6–25)	na
IgM-RF [positive (>25 U/ml)]	n (%)	67 (65)	67 (65)
Joint erosions present [patients]	n (%)	85 (83)	85 (83)
BMI [kg/m^2]	Mean (SD)	25.5 (5)	26.0 (5)
HAQ	Mean (SD)	1.48 (0.62)	1.59 (0.89)
Corticosteroids			
Ever use	n (%)	65 (64)	na
Use [during follow-up]	n (%)	na	58 (57) *
Months used [during follow-up]	Mean (SD)	na	43.8 (25.4)
≥7.5mg for ≥6 months [during follow-up]	n (%0	na	18 (32)
Anti-osteoporosis medication			
Anti-resorptive treatment	n (%)	15 (15)	31 (31)**
Hormone replacement treatment	n (%)	31 (30)	0 (0)**
Calcium supplementation	n (%)	41 (40)	51 (50)**
Vitamin-D supplementation	n (%)	28 (27)	43 (42)**
Vitamin-D and calcium supplementation	n (%)	25 (24)	40 (39)
DMARD treatment [during follow-up]			
Methotrexate	n (%)	na	66 (65)*
Duration [months]	Mean (SD)	na	49.2 (21.7)
Sulfasalazine	n (%)	na	28 (27)*
Duration [months]	Mean (SD)	na	40.3 (25.2)
TNF-inhibitors	n (%)	na	20 (20)*
Duration [months]	Mean (SD)	na	18.2 (11.3)
Other	n (%)	na	44 (43)*
Disease activity			
DAS-28	Mean (SD)	5.4 (1.3)	3.6 (1.2)
ESR [mm/hr]	Median (range)	27 (2–85)	18 (2–93)
CRP [mg/L]	Median (range)	11 (0–175)	5 (9–72)
Mean ESR [mm/hr]	Mean (SD)	na	20.9 (11.8)
Mean CRP [mg/L]	Mean (SD)	na	12.6 (10.9)
Osteoporosis/Osteopenia* **			
Osteoporosis [t-score <-2.5]	n (%)	36 (35)	na
Osteopenia [t-score <-1.5 and >-2.5]	n (%)	26 (26)	na
Fractures			
Vertebral [Genant]	n (%)	15 (25)	32 (33)
Non-vertebral	n (%)	24 (24)	35 (35)

* used for at least 1 month during the 5-year follow-up period.
** using at follow-up.
*** t-scores at either total-hip and/or vertebral spine.
na, non applicable.

The characteristics of the patients during follow-up are shown in Table 1. During follow-up, 58 (57%) patients used corticosteroids for a mean (SD) duration of 43.8 (25.4) months. ART was used by 15% of the patients at baseline and during follow-up an additional 16 patients (16%) started with ART. Calcium and vitamin-D supplementation were ever used by 50% and 42%, respectively for some time during the follow-up period. HRT was used by 31 (30%) patients at baseline, but was discontinued by all patients by the end of the study.

Incident non-vertebral fractures

A total of 18 patients reported 22 fractures. Two patients had fractures due to high-energy trauma (traffic and skiing accident). Thus, 16 (16%) patients had 17 osteoporotic fractures. Fractures were reported at the following anatomical sites: Upper-arm (n=3), wrist (n=4), hip (n=3), upper-leg (n=2), ankle (n=2), ribs (n=2) and pubic bone (n=1). The annual incidence of patients with non-vertebral fractures in our study was 3.2 (95% CI 1.8–5.5) per 100 patients/year.

Incident vertebral fractures

A total of 97 patients had lateral spine X-rays available for evaluation. In a total of 18 (19%) patients 22 new vertebral fractures were identified. All incident fractures occurred in vertebrae which were normal at baseline. Three patients suffered more than one fracture. Most fractures as expected were identified in the mid-thoracic and thoraco-lumbar regions (Figure 1). Fifteen of the 18 patients (83%) had at least a new grade 2 vertebral fracture. The annual incidence rate for a new morphometric vertebral fracture was 3.7 (95% CI 2.2–5.8) per 100 patients/year.

In total 32 (32%) patients had either a new vertebral or a new non-vertebral fracture.

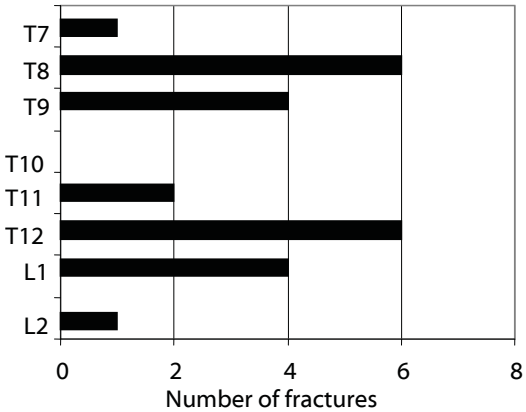

Figure 1 Distribution of new vertebral fractures.

Table 2 Demographics and disease variables for patients with and without new vertebral and non-vertebral fractures baseline or follow-up

		Vertebral fracture			Non-vertebral fracture		
		Yes (18)	No (79)	p	Yes (16)	No (86)	p
Age [years]	mean (SD)	61 (6.5)	60 (5.8)	0.49	62 (5.0)	60 (6.1)	0.20
Disease duration [years]	mean (SD)	17 (8.7)	17 (10.5)	0.95	18 (8.7)	17 (10.7)	0.70
IgM-RF [positive]	n (%)	9 (50)	24 (30)	0.278	10 (62)	57 (67)	0.77
BMI [kg/m^2]	mean (SD)	25.1 (3.8)	25.1 (4.0)	0.96	24.7 (2.9)	25.6 (5.1)	0.27
HAQ	mean (SD)	1.56 (0.35)	1.4 (0.72)	0.30	1.4 (0.75)	1.5 (0.68)	0.79
Use of corticosteroids	n (%)	14 (78)	43 (54)	**0.04**	11 (69)	47 (54)	0.30
Use of ART during follow-up	n (%)	7 (39)	24 (30)	0.49	10 (62)	21 (24)	**0.002**
BMD spine [g/cm^2 at baseline]	mean (SD)	0.981 (0.193)	1.159 (0.516)	0.12	0.969 (0.132)	1.151 (0.585)	0.08
BMD hip [g/cm^2 at baseline]	mean (SD)	0.843 (0.138)	0.840 (0.165)	0.96	0.751 (0.108)	0.858 (0.159)	**0.003**
DAS 28 [at baseline]	mean (SD)	5.2 (0.7)	4.7 (1.2)	0.06	4.8 (1.2)	4.8 (1.2)	0.89
Mean ESR [mm/hr]	mean (SD)	22.3 (13.3)	20.1 (11.5)	0.49	21.7 (13.6)	20.8 (11.6)	0.80
Mean CRP [mg/L]	mean (SD)	15.7 (8.0)	11.3 (8.1)	0.07	12.5 (6.4)	12.7 (11.7)	0.82
Vertebral fracture [at baseline]	n (%)	1 (5)	11 (15)	0.067	5 (31)	19 (22)	0.44
Non-vertebral fracture [at baseline]	n (%)	8 (44)	15 (19)	**0.02**	4 (25)	10 (11)	0.12

Differences in patients with and without vertebral and non-vertebral fractures

On average patients with a new non-vertebral fracture had a lower BMD at baseline compared to patients without a new non-vertebral fracture. This was significant only for the baseline BMD at the hip ($p<0.05$). In the group of patients with a new non-vertebral fracture more patients used ART than in the group without a new non-vertebral fracture (62% versus 24%, $p<0.05$).

When comparing patients with and without incident vertebral fractures, there were significantly more patients using corticosteroids (78% versus 54%) in the patients with a new vertebral fracture during follow-up ($p<0.05$). Patients with new vertebral fractures had also suffered significantly more non-vertebral fractures at baseline ($p<0.05$), but seemed to have less vertebral fractures at baseline ($p=0.067$). There was also a trend for a higher disease activity (mean CRP during follow-up and DAS-28 at baseline) in the patients with a new vertebral fracture compared to patients without a new vertebral fracture (Table 2).

Of the patients who were osteopenic at baseline 7 (19%) sustained a new vertebral fracture and 6 (17%) a new non-vertebral fracture during follow-up. In the group of osteoporosis patients there were 7 (27%) new vertebral and 7 (27%) new non-vertebral fractures during follow-up.

Possible risk factors for incident fractures

In the multivariate logistic analysis, we identified BMD at the total-hip as an independent predictor for incident non-vertebral fractures. BMD of the spine, ever steroid use, vertebral fractures and non-vertebral fractures at baseline were entered into the model, but were eliminated as not significant (Table 3). Non-vertebral fractures at baseline were an independent predictor of new vertebral fractures. BMD of the spine,

Table 3 Multivariate analyses of incident fractures

	B	OR (95%CI)	p
	Non-vertebral fractures		
BMD total-hip [(1,0 g/cm^2]	-5.6	0.003 (0.001–0.42)	0.019
Constant	2.8	16.1	0.133
	Vertebral fractures		
Non-vertebral fracture at baseline	1.21	3.4 (1.3–9.6)	0.029
Constant	0.6	1.8	0.54

mean CRP over the follow-up period, DAS-28 at baseline and ever steroid use were entered into the model but were eliminated (Table 3). All regression models were corrected for centre.

DISCUSSION

In this 5-year follow-up study of postmenopausal women with established RA, we found a high incidence of vertebral and non-vertebral fractures. Baseline non-vertebral fractures were an independent predictor of new vertebral fractures and new non-vertebral fractures were independently predicted by baseline BMD at the hip. This is the first study to study incident non-vertebral fractures and morphometric vertebral fractures in RA in a single study. These data are also unique because of the duration of follow-up (5 years).

In total, 19% of the patients had a new vertebral fracture during the 5-year follow-up, corresponding to an annual incidence of 3.7/100 patients/year. Because this is an observational study, we have no data from a control group to compare this annual incidence to. Comparison with other historical cohorts is possible. In the EPOS study, a study of fractures in the general population of 50 years and older, the annual incidence rate of morphometric vertebral fractures in females was 1.07/100 patient years [13]. Mean age (63 years) for these patients is comparable to our study. In another study by Nevitt et al. the annual incidence of morphometric fractures was 0.8/100 patient years. This study assessed fractures in subjects 65 years and older from the general population [14]. Although comparisons between studies should be considered with caution, these studies give a clear indication of the high incidence rate of vertebral fractures in our study. The vertebral fractures we found were also predominantly moderate and severe fractures (grades II and III). There are 2 studies which performed a longitudinal study on radiological detected vertebral fractures. Ørstavik et al. found 6.7 incident deformities per 100 patient years in a group of 255 female RA patients (mean age 54.3 years) during a mean follow-up of 2.3 years [15]. This study however did not use vertebral spine-X-rays but morphometric X-ray absorptiometry, this different technique may explain the higher incidence rate of vertebral fractures in this otherwise comparable study. In the other study, Katsumitsu et al. [16] found new vertebral fractures in 19 (16%) patients out 112 patients followed for 4 year. This percentage is comparable to the percentage of vertebral fractures found in our study during 5 years (19%). Unfortunately no data is given on the mean disease duration and mean age of the included RA patients.

In our study we also found a high frequency of non-vertebral fractures. When comparing our annual incidence of 3.1/100 patient years with the incidence from the

female population in the EPOS study 1.9/100 patient years, it is considerably higher. The EPOS is a study investigating limb fractures in man and women aged 50 tot 79 years [17]. Finigan et al. also found an incidence 1.9 of new vertebral fractures per 100 patient years in a 10 year follow-up population based study. Three hundred seventy-six female patients were included into this study with an age (64.6 years) at baseline which is comparable to our cohort [18]. Few studies have investigated the incidence of clinical fractures in RA patients. In a large database study by van Staa et al., they identified an increased risk of fractures of 1.5 for all fractures in RA patients compared to healthy controls [4]. This study included all clinical fractures, also including clinical vertebral fractures. Nampei et al. found in a cohort of 209 RA patients (86% female, mean age 60 years) an incidence of patients with new fractures of 11.5/100 patient years [19]. This is a very high incidence, but this study investigated all patients with pain suspicious of a fracture very thoroughly (including MRI) for fractures, which could very well explain the high incidence of fractures in this study.

In our study we found few risk factors for new fractures. Our study only revealed well known risk factors for new vertebral fractures and new non-vertebral fractures, respectively baseline non-vertebral fractures and BMD of the hip at baseline. We did not find any specific RA related factors to be predictors for new fractures. Mean CRP and baseline DAS-28 showed a trend to be increased in patients with a new vertebral fracture (Table 3), but were not independent predictors of future vertebral fractures.

Our study has several limitations. We performed measurements at baseline and at follow-up at 5 years. This is a quite long period and measurements like DAS-28 at baseline and follow-up will probably not properly reflect the fluctuation of the disease activity during that period. This could explain why we found no associations between fractures and disease activity. Another reason for not finding an association could be that joint scores were performed by different investigators, which can cause some variability in measurements. However we also did not find an association with objective disease activity measures like CRP and ESR. Finally, our studied population might also be too small to find risk factors in rheumatoid arthritis for a multifactorial disease like osteoporotic fractures. Another limitation of having only measurements at baseline and 5 years is, that we were not able to properly investigate the relationship between timing of the use of anti-resorptive treatment and new fractures especially vertebral fractures. It could be very likely that bisphosphonates were started after a fracture occurred and this is probably the reason is why we did not find a protective effect of bisphosphonates for example.

In conclusion, in our study we found a high incidence rate of vertebral and non-vertebral fracture rates during a follow-up of 5 years in patients with established RA compared to the general population.

REFERENCES

1 Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum 2000; 43:522-30.

2 Lems WF, Dijkmans BA. Should we look for osteoporosis in patients with rheumatoid arthritis? Ann Rheum Dis 1998; 57:325-7.

3 Cooper C, Coupland C, Mitchell M. Rheumatoid arthritis, corticosteroid therapy and hip fracture. Ann Rheum Dis 2000; 54:49-52.

4 van Staa TP, Geusens P, Bijlsma JW, Leufkens HG, Cooper C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54:3104-12.

5 Ørstavik RE, Haugeberg G, Mowinckel P, Høiseth A, Uhlig T, Falch JA, Halse JI, McCloskey E, Kvien TK. Vertebral deformities in rheumatoid arthritis: a comparison with population-based controls. Arch Intern Med 2004; 23:420-5.

6 Burger H, van Daele PL, Algra D, Hofman A, Grobbee DE, Schütte HE, Birkenhäger JC, Pols HA. Vertebral deformities as predictors of non-vertebral fractures. BMJ 1994; 309: 991-2

7 Lindsay R, Silverman SL, Cooper C, Hanley DA, Barton I, Broy SB, Licata A, Benhamou L, Geusens P, Flowers K, Stracke H, Seeman E. Risk of new vertebral fracture in the year following a fracture. JAMA 2001; 285:320-3

8 Lodder MC, Haugeberg G, Lems WF, Uhlig T, Orstavik RE, Kostense PJ, Dijkmans BA, Kvien TK, Woolf AD; Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Radiographic damage associated with low bone mineral density and vertebral deformities in rheumatoid arthritis: the Oslo-Truro-Amsterdam (OSTRA) collaborative study. Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Arthritis Rheum 2003; 49:209-15

9 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

10 Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980; 23:137-45.

11 van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39:34-40.

12 Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res 1993; 8:1137-48

13 EPOS study group. Incidence of vertebral fracture in europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res 2002; 17:716-24

14 Nevitt MC, Cummings SR, Stone KL, Palermo L, Black DM, Bauer DC, Genant HK, Hochberg MC, Ensrud KE, Hillier TA, Cauley JA. Risk factors for a first-incident radiographic vertebral fracture in women at least 65 years of age: the study of osteoporotic fractures. J Bone Miner Res 2005; 20:131-40.

15 Ørstavik RE, Haugeberg G, Uhlig T, Mowinckel P, Falch JA, Halse JI, Kvien TK. Incidence of vertebral deformities in 255 female rheumatoid arthritis patients measured by morphometric X-ray absorptiometry. Osteoporos Int 2005; 16:35-42.

16 Arai K, Hanyu T, Sugitani H, Murai T, Fujisawa J, Nakazono K, Kondo N, Endo N. Risk factors for vertebral fracture in menopausal or postmenopausal Japanese women with rheumatoid arthritis: a cross-sectional and longitudinal study. J Bone Miner Res 2006; 24:118-24.

17 Ismail AA, Pye SR, Cockerill WC. Incidence of limb fracture across Europe: results from the European Prospective Osteoporosis Study (EPOS). Osteoporos Int 2002; 13:565-7.

18 Finigan J, Greenfield DM, Blumsohn A, Hannon RA, Peel NF, Jiang G, Eastell R. Risk factors for vertebral and nonvertebral fracture over 10 years: a population-based study in women. J Bone Miner Res 2008; 23:75-85.

19 Nampei A, Hashimoto J, Koyanagi J, Ono T, Hashimoto H, Tsumaki N, Tomita T, Sugamoto K, Nishimoto N, Ochi T, Yoshikawa H. Characteristics of fracture and related factors in patients with rheumatoid arthritis. Mod Rheumatol 2008; 18:170-6.

Chapter 3b

Generalised bone loss during 5 years in female rheumatoid arthritis patients with established disease

M. Vis
P. Bøyesen
M. Hoff
E.A. Haavardsholm
G. Haugeberg
T. Uhlig
A. Woolf
B. Dijkmans
T.K. Kvien
W.F. Lems

Submitted

ABSTRACT

Objective: To investigate the changes over 5 years in BMD of the spine and hip.

Methods: 102 female RA patients form the original OSTRA-study participated in the follow-up study. The bone mineral density (BMD) was measured at baseline and after 5 years.

Results: There was a significant decrease of 5.9% ($p<0.05$) in total-hip BMD (g/cm^2). At the spine (L2–L4) there were a trend toward a loss (-2.4%, $p=0.059$).

Conclusion: We found that even in a postmenopausal population with established, there was a substantial decrease in BMD of 5.9% at the hip and a 2.4% bone loss at the spine.

INTRODUCTION

Osteoporosis occurs more frequently in patients with rheumatoid arthritis (RA) than in the healthy population. In cross-sectional studies, the prevalence of osteoporosis (low BMD) is increased in RA patients compared to the general population, while in other studies also elevated vertebral and non-vertebral fracture rates have been shown [1-5]. Longitudinal studies have linked increased bone loss in RA to disease related factors such as disease activity, disease duration, immobility and the use of glucocorticoids [6-9].

In a previous, multi-centre cross-sectional study of the OSTRA group we found that high age, low body mass index, high cumulative dose of corticosteroids were related to low bone mineral density (BMD) at the lumbar spine and hip and that radiological joint damage in RA was associated with a low BMD at the hip [9]. In the present study, we investigated the longitudinal changes over 5 years in BMD of the spine and hip and the factors influencing BMD in our cohort of postmenopausal RA patients.

PATIENTS AND METHODS

Patients

The 150 female RA patients from the original study were asked to participate in the follow-up study at 5 years [9, 10]. In total, 102 patients of the original cohort agreed to a follow-up assessment (35 from Oslo, 34 from Truro and 36 from Amsterdam). There were no significant differences in baseline characteristics between patients with follow-up measurements and withdrawals (data not shown).

Data

The baseline data collection is described elsewhere [9]. At 5-year follow-up the patients completed questionnaires, underwent clinical examination, and blood samples were taken. Calcium intake, length and weight, use of disease modifying anti-rheumatic drugs (DMARDs), anti-osteoporotic drugs, and corticosteroids during the last 5 years were recorded.

Bone mineral density measurement of hip and spine

The bone mineral density (BMD) was measured at baseline and after 5-year follow-up. All measurements were performed on the same DXA machines as used in the baseline study.

Statistical analysis

Statistical analyses were performed using the SPSS (Chicago, IL), version 15.0.

Independent group differences were tested by two-sided t-tests for continuous variables and chi-square tests for counts. Possible predictors of bone loss were explored in univariate regression analyses and associations with $p<0.2$ were subsequently entered into a multivariate linear regression analysis (backward procedure), applying BMD change during follow-up as the dependent variable. Standard diagnostic tests were performed, all tests were two-sided and $p<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Patient characteristics at baseline and 5-year follow-up are presented in Table 1.

Changes in BMD

There was a significant decrease of 5.9% ($p<0.05$) in total-hip BMD (g/cm^2) from 0.843 (0.156) to 0.793 (0.151). At the spine (L2–L4) there were a trend toward a loss (-2.4%, $p=0.059$) from mean BMD (g/cm^2) 1.045 (0.190) at baseline to 1.020 (0.187) at 5 years. In Figure 1A and 1B probability plots display 5-year BMD for all patients and per center. Patients in Amsterdam tended to loose more bone at the spine than in Oslo and Truro ($p<0.05$).

Determinants of bone loss

In the prediction model changes in BMD use of anti-resorptives was the only independent predictor for changes in BMD of the spine and hip (Table 2). Daily calcium intake, cumulative prednisone use, baseline BMD, baseline HAQ and disease duration were also entered into the model, but did not contribute significantly to the model when correcting for age and center.

DISCUSSION

In this observational study over 5 years we found a significant decrease in BMD at the total-hip of 5.9%, while at the lumbar spine there was a strong trend towards bone loss (-2.4%, $p=0.06$). This is a remarkably high rate of generalized bone loss, especially at

Table 1 Characteristics of the 102 patients

Baseline		
Age [years]	Mean (SD)	61 (6)
Disease duration [years]	Median (range)	17 (6-25)
IgM-RF [positive]	n (%)	67 (65)
DAS-28	Mean (SD)	5.4 (1.3)
HAQ	Mean (SD)	1.48 (0.62)
Follow-up		
DAS-28	Mean (SD)	3.6 (1.2)
HAQ	Mean (SD)	1.59 (0.89)
Use of corticosteroids		
Used*	n (%)	65 (64)
Mean cumulative dose [mg]	Mean (SD)	2,900 (1,800)
Osteoporosis medication		
Anti-resorptives*	n (%)	57 (56)
Bisphosphonates*	n (%)	31 (30)
HRT*§	n (%)	31 (30)
Calcium supplementation*	n (%)	51 (50)
Vitamin-D supplementation*	n (%)	43 (42)
Use of DMARDs		
MTX*	n (%)	66 (65)
SASP*	n (%)	28 (27)
Anti-TNF*	n (%)	20 (20)
Other*	n (%)	44 (43)
Disease activity		
Mean ESR [mm/hr during study]	Median (range)	20.9 (11.8)
Mean CRP [mg/l during study]	Median (range)	12.6 (10.9)

* Used for some time (> 1 month) during the studied period.
§ All patients had discontinued HRT before the end of the study.

Table 2 Predictors of BMD change at hip and spine corrected for age and center

	B	SE	p
$R^2 = 0.09$	Change in hip BMD		
Anti-resorptive drugs	0.032	0.013	0.015
Constant	-0.021	0.068	
$R^2 = 0.11$	Change in spinal BMD		
Anti-resorptive drugs	0.086	0.027	0.035
Constant	-0.303	0.142	

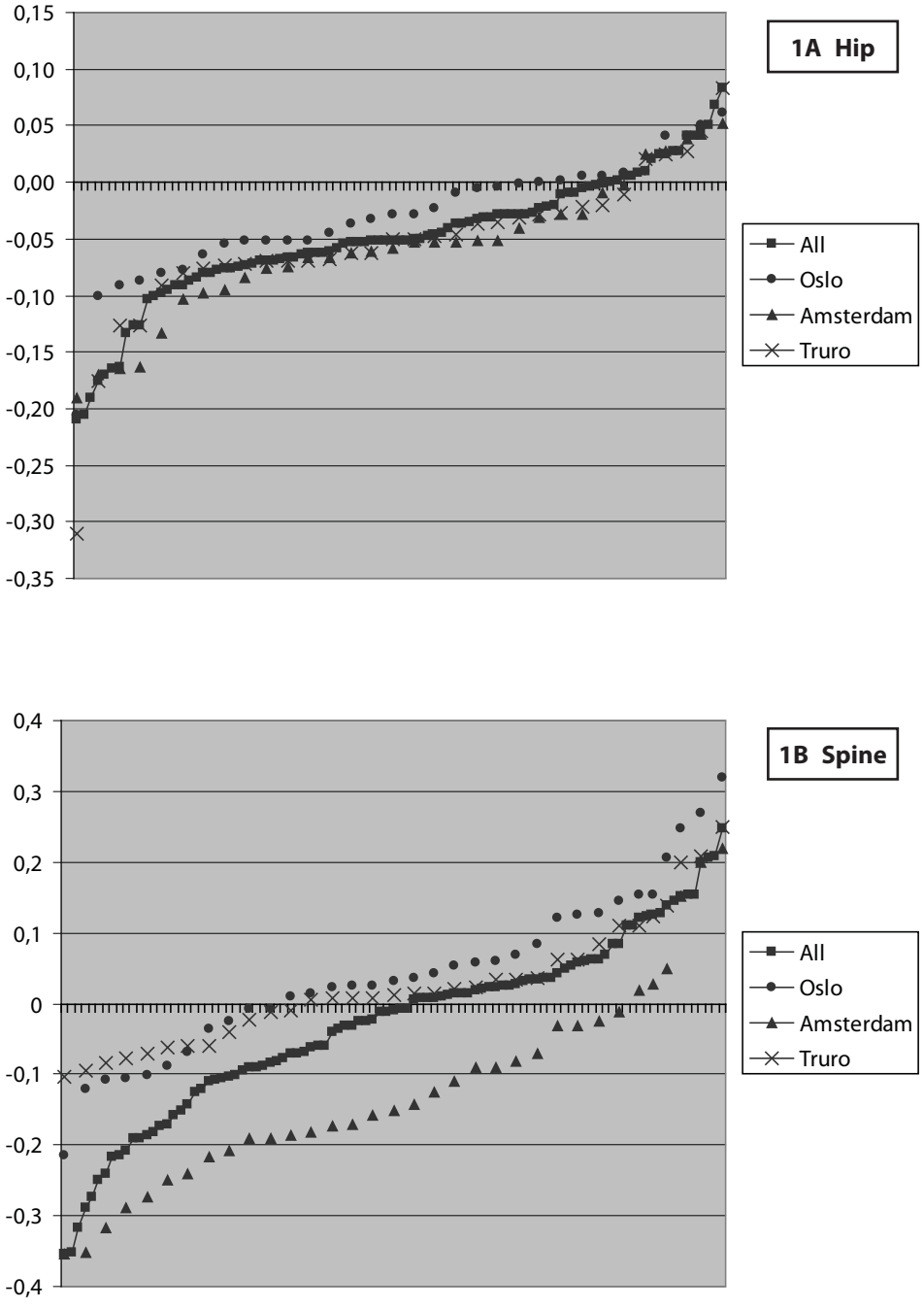

Figure 1A and 1B Probability plot of the change of BMD (g/cm^2) at hip and spine during the 5-year follow-up for all patients and per center.

the hip, during a time period in which treatment of RA is dramatically improved by the use of combination therapy with conventional drugs or biologicals, for instance TNF-blockers [13].

We compared our data to other studies investigating changes in BMD: in a study by Shibuya et al. a decrease in spinal BMD of 1% during 1 year follow-up in an unselected cohort of 146 RA patients was found [8]. Haugeberg et al. also investigated the change of BMD in a population based cohort of RA patients and found a decrease of -0.77% at the total hip to -0.29% at the spine during 2 years [6]. In this study patients using HRT or bisphosphonates had a lower rate of bone loss. Studies excluding patients with anti-osteoporotic treatment tend to find a higher rates of bone loss, for example Cortet et al. found a loss of 2.1% at the spine and 3.1 at the hip in a cohort of RA patients during 18 months follow-up [7]. As in our study the bone loss seems to be greater at the hip than that at the spine.

When we compare our data to an osteoporotic female population without RA of comparable age, we found a higher rate of generalized bone loss in our study; for example the placebo arm of a randomized control trial investigating the effect of treatment with alendronate in postmenopausal women, these patients showed non-significant decreases in BMD of the spine and hip of respectively 0.6% and 0.7% during 3 years [10].

Several studies have shown that active treatment of RA limits BMD loss. In a previous 1 year study in RA patients treated with anti-TNF we showed that there was no loss of bone at hip and spine, this was especially true for the group of patients who had a good clinical response on anti-TNF. In this study we did not find an association between BMD loss and disease activity markers or treatment with anti-TNF drugs. Only use of anti-resoprtives (bisphosphonates and HRT) was an independent predictor of change in BMD at the hip or spine. It is difficult to investigate factors associated with BMD changes in RA patients because of the multifactorial nature of both diseases and the possible interactions between variables (i.e. corticosteroids suppress disease activity but also decrease BMD). This could be one of the reasons why we found so few predictors for change in BMD. Another reason of course could be due to the size of the cohort.

In conclusion this is one of the first studies investigating BMD changes in RA patients over a long period (5 years). We found that even in a postmenopausal population with established RA of which one third was treated with bisphosphonates, there was still a substantial decrease in BMD of 5.9% at the hip and a 2.4% bone loss at the spine.

REFERENCES

1 Haugeberg G, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: results from 394 patients in the Oslo County Rheumatoid Arthritis register. Arthritis Rheum 2000; 43:522-30.

2 Lane NE, Pressman AR, Star VL, Cummings SR, Nevitt MC. Rheumatoid arthritis and bone mineral density in elderly women. The Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1995; 10:257-63.

3 Magaro M, Tricerri A, Piane D, Zoli A, Serra F, Altomonte L, Mirone. Generalized osteoporosis in non-steroid treated rheumatoid arthritis. Rheumatol Int 1991; 11:73-6.

4 van Staa TP, Geusens P, Bijlsma JW, Leufkens HG, Cooper C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54:3104-12.

5 Ørstavik RE, Haugeberg G, Mowinckel P, Høiseth A, Uhlig T, Falch JA, Halse JI, McCloskey E, Kvien TK. Vertebral deformities in rheumatoid arthritis: a comparison with population-based controls. Arch Intern Med 2004; 164:420-5.

6 Haugeberg G, Ørstavik RE, Uhlig T, Falch JA, Halse JI, Kvien TK. Bone loss in patients with rheumatoid arthritis: results from a population-based cohort of 366 patients followed up for two years. Arthritis Rheum 2002; 46:1720-8.

7 Cortet B, Guyot MH, Solau E, Pigny P, Dumoulin F, Flipo RM, Marchandise X, Delcambre B. Factors influencing bone loss in rheumatoid arthritis: a longitudinal study. Clin Exp Rheumatol 2000; 18:683-90.

8 Shibuya K, Hagino H, Morio Y, Teshima R. Cross-sectional and longitudinal study of osteoporosis in patients with rheumatoid arthritis. Clin Rheumatol 2002; 21:150-8.

9 Lodder MC, Haugeberg G, Lems WF, Uhlig T, Ørstavik RE, Kostense PJ, Dijkmans BA, Kvien TK, Woolf AD; Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Radiographic damage associated with low bone mineral density and vertebral deformities in rheumatoid arthritis: the Oslo-Truro-Amsterdam (OSTRA) collaborative study. Arthritis Rheum 2003; 49:209-15.

10 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

11 van Gestel AM, Prevoo ML, 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39:34-40.

12 Devogelaer JP, Broll H, Correa-Rotter R, Cumming DC, De Deuxchaisnes CN, Geusens P, Hosking D, Jaeger P, Kaufman JM, Leite M, Leon J, Liberman U, Menkes CJ, Meunier PJ, Reid I, Rodriguez J, Romanowicz A, Seeman E, Vermeulen A, Hirsch LJ, Lombardi A, Plezia K, Santora AC, Yates AJ, Yuan W. Oral alendronate induces progressive increases in bone mass of the spine, hip, and total body over 3 years in postmenopausal women with osteoporosis. Bone 1996; 18:141-50.

13 van Vollenhoven RF. Treatment of rheumatoid arthritis: state of the art 2009. Nat Rev Rheumatol 2009; 5:531-41.

Chapter 3c

High disease activity is a predictor of cortical hand bone loss in postmenopausal patients with established rheumatoid arthritis: A 5-year multicentre longitudinal study

M. Hoff*
P. Bøyesen*
G. Haugeberg
M. Vis
A.D. Woolf
E.A. Havaardsholm
B.A.C. Dijkmans
T.K. Kvien
T. Uhlig
W.F. Lems
*Shared first authorship

Rheumatology (Oxford) 2010 Sep; 49(9):1676-82

ABSTRACT

Objective: The objective of this study was to examine 5-year change in cortical hand bone mineral density (BMD) in female rheumatoid arthritis (RA) patients with established disease. Further, possibly baseline predictors of 5-year loss in DXR-BMD were studied.

Methods: This 5-year multicenter, longitudinal study included patients from Amsterdam (the Netherlands), Truro (UK) and Oslo (Norway). At baseline 50 patients were consecutively included per centre. Inclusion criteria were: Female sex, age 50–70 years and disease duration ≥ 5 years. This study presents 5-year follow-up data for 85 of these 150 patients (29 from Amsterdam; 26 from Truro; and 30 from Oslo). Clinical examination, blood test and radiographs were taken at baseline and 5-year follow-up. Cortical hand BMD was measured by Digital X-ray radiogrammetry (DXR) from hand radiographs.

Results: The mean (95% CI) baseline DXR-BMD for all patients was 0.46 g/cm^2 (0.44 to 0.48) and the median 5-year DXR-BMD change was -6.7% (-11.2 to -2.82). 5-year DXR-BMD loss was associated with baseline measurements of age, RF, CRP, HAQ and DAS-28 in univariate linear regression analyses. DAS-28 at baseline was an independent predictor of 5-year DXR-BMD loss in multivariate linear regression analyses corrected for centre, age and use of bone protective agents.

Conclusion: High disease activity measured by DAS-28 was an independent predictor of cortical hand bone loss over 5 years in established, destructive RA. This finding supports that increased disease activity leads to localised bone loss in longstanding RA and underlines the importance of tight control and aggressive anti-inflammatory treatment in this patients.

INTRODUCTION

Periarticular bone loss is an early characteristic feature of rheumatoid arthritis (RA) visible on radiographs and together with erosions considered a hallmark of the disease [1]. Inflammatory activation of the osteoclast is involved in both features. Studies support that cytokines such as TNFα, interleukin-1 (IL-1), IL-6, macrophage colony-stimulating factor and receptor activator of nuclear factor-κ ligand (RANKL), activate the osteoclast which causes osteoporosis (localised and generalised) and erosions [2-4].

Quantitative hand bone loss can be measured by dual energy X-ray absorptiometry (DXA) bone mineral density (BMD) which measure the total amount of local bone [5] and digital X-ray radiogrammetry (DXR) which measures cortical bone [6]. The advantage of DXR is that BMD can be analysed from hand radiographs, often taken routinely of RA patients in daily clinical practice. Previous studies suggest that DXA measured bone loss only takes place the first 2-3 years after disease start [7-9], while one study suggest that DXR measured loss occur through the whole disease course [9]. However, there is limited information concerning hand bone loss in established RA. Inflammation of the joints is not restricted to the early phase of the RA disease, but may be present during the entire disease course [10]. Hence, theoretically one could consider applying hand bone as an outcome measure also in established RA.

The main objective of this multi-centre observational study was to examine 5-year change in hand DXR-BMD in RA patients with established disease and to possibly identify baseline predictors of 5-year change in DXR-BMD.

METHODS

Patients

The original cohort consisted of 150 women with established RA who were recruited from rheumatology clinics in Amsterdam, the Netherlands; Truro, UK; and Oslo, Norway. At baseline 50 patients were consecutively included per centre and inclusion criteria were: female sex, age 50–70 years and RA disease duration ≥ 5 years [11]. Hand BMD measured with DXR was performed in 135 of the original cohort at baseline [12]. We now present 5 year follow-up data for DXR-BMD for 85 of these patients (29 from Amsterdam; 26 from Truro; and 30 from Oslo). A flow-chart of the patients is depicted in Figure 1. Disease activity at baseline was assessed by joint counts (28-tender and 28-swollen joint counts), patient and physician's global assessment of disease activity and by acute phase reactants. The disease activity score DAS-28 was computed based on erythrocyte sedimentation rate (ESR) [13].

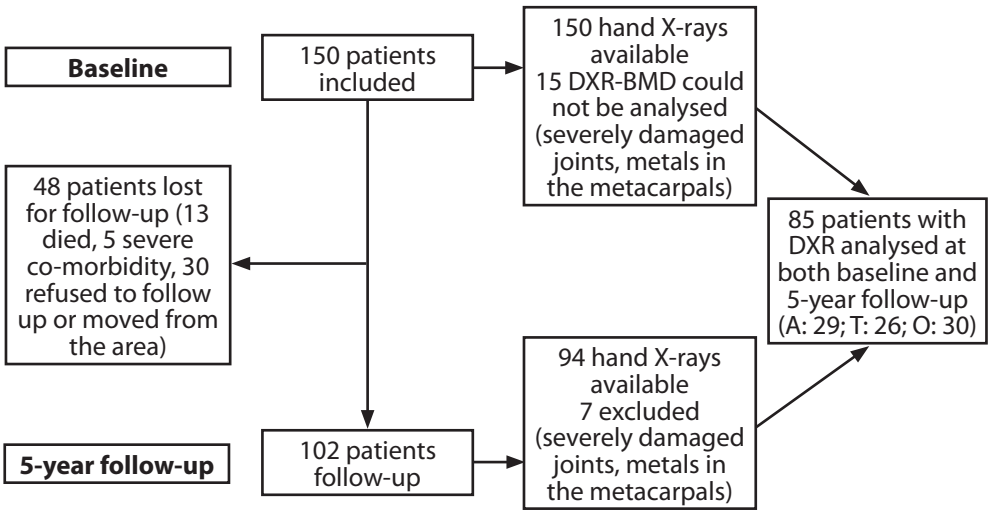

Figure 1 Flow-chart of the included patients in the present study. A, Amsterdam; T, Truro; O, Oslo.

Radiographs and DXR-BMD analyses

DXR (Sectra, Linköping, Sweden) was used to measure hand BMD on bilateral hand radiographs taken in postero-anterior view. DXR is a computer version of the traditional radiogrammetry technique [14] and the method has previously been described in detail [6]. On hand radiographs, the computer automatically recognizes regions of interest around the narrowest part of the second, third, and fourth metacarpal bone and measure cortical thickness, bone width and porosity 118 times per cm. DXR-BMD is defined as $c \; X \; VPA_{comb} \; X \; (1\text{-}p)$, where c is a density constant, VPA is cortical volume per area, and p is porosity. To avoid bias regarding dominant and non-dominant hands and to achieve better precision, we used the mean of both hands [15]. If the radiograph from one hand could not be analysed, we used the radiograph from the available hand for analyses at both time points. At baseline all radiographs were conventional films and DXR-BMD were analysed with Pronosco X-posure 2.0. Short-time precision (coefficient of variation, CV%) for Pronosco X-posure based on duplicate hand radiographs with reposition between each measurement in healthy individuals has been found to be 0.28% for mean of both hands [9]. At 5-years follow-up DXR-BMD for the radiographs from Amsterdam (conventional) and Truro (digitised and printed out) were analysed with the same Pronosco X-posure 2.0 equipment, while the radiographs from Oslo were digitised and analysed with dxr-online (Sectra, Sweden). The same algorithm was used for calculating DXR-BMD both in Pronosco X-posure and dxr-online. However, analysing digitised radiographs has been shown to be more influenced by

the radiographic equipment than conventional radiographs [16-18]. Seven patients' radiographs failed DXR-BMD analyses due to severely damaged joints or metal implants at 5-year follow-up. These patients showed a trend toward a longer disease duration (20 vs. 13.5 years, p=0.12) and older age (59.6 vs. 62.4 years, p=0.27).

The baseline radiographs of the hands and wrists were scored according to the van der Heijde modified Sharp score (vdHSS) by a trained observer (PB) [19].

Statistical methods

The analyses were performed using SPSS 14 statistics package (SPSS Inc, USA). Baseline characteristics were described using median and inter quartile range (IQR) for continuous variables and percentage for counts. Independent groups were compared using Mann-Whitney U test for continuous variables, χ^2-tests for dichotomous variables and Kruskall Wallis for differences between centres. DXR-BMD change was calculated as the percentage difference between the follow-up value and the baseline value and bone loss over time was expressed as a negative value. The individual 5-year DXR-BMD changes were depicted in cumulative probability plots [20].

Possible predictors for 5-year DXR-BMD loss were examined by univariate linear regression analyses. The independent variables with a p-value ≤ 0.20 were included in multivariate linear analyses with 5-year changes in DXR-BMD as the dependent variable. The final multivariate model was obtained by enter procedure and standard diagnostic tests of model assumptions and residuals were routinely performed.

All tests were two-sided and p-values ≤ 0.05 were considered to be statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics comparing patients with and without 5-year DXR-BMD measurements are presented in Table 1, and the patient flow is presented in Figure 1. The 85 included patients were younger, had a significantly lower ESR, HAQ and they also had significantly higher DXA of the total hip compared with those who were not included (n=65) from the original study (Table 1). The two groups were comparable regarding disease duration, positive rheumatoid factor, DAS-28 and use of DMARDs, prednisolone and bone protective treatment. There were no significant differences in the number of patients from the different centres available for follow-up at 5 years (29 from Amsterdam; 26 from Truro; and 30 from Oslo, p=0.88, χ^2-tests).

Table 1 Baseline characteristics of female patients with rheumatoid arthritis with and without hand bone density measurements (Median (IQR) or absolute value (percent))

	Patients with DXR-BMD measurements n=85	Patients without DXR-BMD measurements n=65	p-value†
Demographic variables			
Age (years)	59.7 (54.8–64.3)	64.0 (58.8–67.9)	<0.01*
Menopause	74 (87%)	64 (99%)	0.01*
BMI (kg/m^2)	25.5 (22.8–27.4)	24.3 (22.6–27.8)	0.78
Smoker (current)	24 (28%)	24 (37%)	0.40
Disease duration (years)	13.5 (9.5–21.6)	16.5 (10.0–23.0)	0.46
HAQ (range 1–4)	1.50 (0.88–1.88)	1.87 (1.14–2.25)	0.01*
Disease variables			
Rheumatoid factor positive	51 (60%)	43 (66%)	0.44
ESR (mm/hour)	18.0 (10.0–29.0)	23.5 (13.0–40.8)	0.03*
DAS-28	4.8 (4.1–5.6)	5.0 (4.1–5.8)	0.43
Radiographic damage (vdHSS)	16 (5–42)	NA	
Medication at baseline			
DMARDs	62 (73%)	46 (71%)	0.46
Corticosteroid	28 (33%)	29 (45%)	0.19
Bone protective agents	51 (60%)	39 (60%)	1.00
Medication during follow-up			
Anti-TNFα therapy	16 (19%)	NA	
Corticosteroid	40 (47%)	NA	
Bone protective agents	44 (52%)	NA	

† Mann-Whitney U for continuous variables, χ^2-tests for categorical variables; * p≤0.05. IQR, inter quartile range; BMI, body mass index; HAQ, health assessment questionnaire; ESR, erythrocyte sedimentation rate; DAS-28, disease activity score; vdHSS, van der Heijde Sharp score; DMARDs, disease modifying anti-rheumatic drugs; Bone protective agents, use of estrogens or bisphosphonates during follow-up.

Baseline and follow-up treatment are summarised in Table 1. Overall, 52% of the patients used bone protective treatment (defined as bisphosphonates, estrogens or raloxifen) during follow-up. The patients receiving bone protective treatment at baseline had similar DAS-28 as those who did not (4.8 vs. 4.5, p=1.0). None of the patients were treated with teriparatide or calcitonin. Anti-TNFα therapy was used by 19% of the patients.

DXR-BMD change during the follow-up period

The mean (95% CI) baseline DXR-BMD for all patients was 0.46 g/cm^2 (0.44 to 0.48) and the median (IQR) 5-year DXR-BMD change was -6.7% (-11.2 to -2.82). The individual 5-year DXR-BMD losses stratified for centre are depicted in a cumulative

probability plot (Figure 2). The median (IQR) 5-year DXR-BMD change in Amsterdam, Truro and Oslo were -5.1% (-10.7 to -2.4), -8.1% (-12.4 to -5.2) and -6.9% (-10.7 to -2.8), respectively. There were no statistically significant differences between the centres (p=0.19, Kruskall Wallis test). The vdHSS change during the 5-year follow-up period was median 8 units (interquartile range 0–16.5). The correlation between 5-year change in hand DXR-BMD and vdHSS was -0.24 (p=0.04).

Associations between 5-year DXR-BMD change and baseline characteristics and prediction of hand bone loss

The associations between 5-year change in DXR-BMD and baseline variables and treatment during follow-up are presented in Table 2. Five-year DXR-BMD loss was significantly associated with lower age, positive RF, elevated CRP, HAQ and DAS-28 in univariate linear regression analyses.

Table 2 Associations between 5-year DXR-BMD change (follow-up minus baseline) and baseline characteristics as well as treatment during follow-up (univariate linear regression analyses)

		B	95% CI	p-value
Center†	**Oslo**	**-1.2**	**-4.7 – 2.3**	**0.50**
	Truro	**-3.2**	**-6.8 – 0.4**	**0.08**
Age		**0.2**	**-0.01 – 0.5**	**0.06**
Age (grouped into 50–59 vs. 60–70)		**3.8**	**1.0 – 6.6**	**0.009***
Disease duration (years)		-0.04	-0.2 – 0.1	0.60
BMI (kg/m^2)		0.1	-0.3 – 0.5	0.60
Swollen joints		**-0.3**	**-0.6 – 0.02**	**0.06**
Tender joints		**-0.2**	**-0.4 – 0.05**	**0.12**
ESR (mm/hr)		-0.1	-0.1 – 0.04	0.30
CRP (mg/L)		**-0.1**	**-0.2 – -0.02**	**0.02***
RF (negative vs. positive)		**-3.5**	**-6.5 – 0.4**	**0.02***
HAQ		**-2.1**	**-4.3 – -0.02**	**0.05**
DAS-28		**-2.0**	**-3.2 – -0.7**	**0.002***
Baseline DXR-BMD		-8.2	-26.3 – 9.8	0.37
Prednisolone during follow-up (yes/no)		-0.4	-11.3 – 10.6	0.95
Total dose of Prednisolone (g)		$4x10^{-5}$	0.0 – 0.0	0.42
Anti-TNFα therapy during follow-up (yes/no)		0.7	-3.2 – 4.5	0.73
Duration of anti-TNF-α therapy (months)		0.01	-0.1 – 0.1	0.85
Bone protective agent during follow-up		**2.3**	**-0.7 – 5.4**	**0.13**

† Amsterdam used as reference. * p-value ≤ 0.05. Covariates eligible for multivariate analyses are marked in bold. BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; HAQ, health assessment questionnaire; DAS-28, disease activity score based on 28 joint count; DXR-BMD, digital X-ray radiogrammetry; TNFα, tumour necrosis factor α.

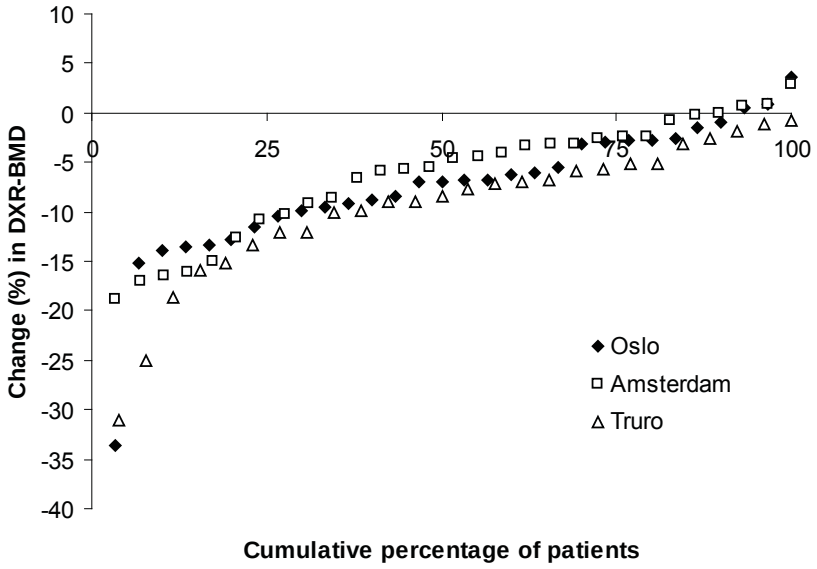

Figure 2 Cumulative probability plot of 5-year DXR-BMD change (%) stratified by centre.

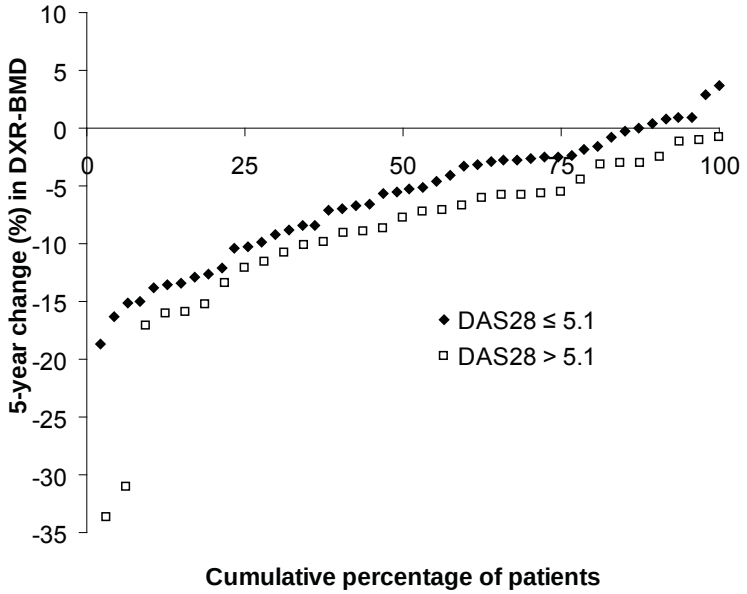

Figure 3 Cumulative probability plots of 5-year DXR-BMD change (%), stratified for high disease activity (DAS-28 > 5.1) versus moderate/low disease activity (DAS-28 ≤ 5.1).

Baseline DAS-28 was maintained as an independent predictor of 5-year change in DXR-BMD in the final multivariate linear regression model. The final model explained 31% of the variation (R^2) in DXR-BMD change and was corrected for age, centre and use of bone protective agents. The effect of disease activity on hand bone loss is illustrated by a cumulative probability plot depicting the 5-year DXR-BMD change stratified for high (DAS-28 > 5.1) versus low and moderate disease activity (Figure 3). DAS-28 of 5.1 is the cut point between moderate and high disease activity and was therefore selected as a cut-off for high disease activity [21].

DISCUSSION

The main finding in this study was that baseline disease activity independently predicted hand BMD loss in postmenopausal patients with established, severe RA. Only few studies have examined associations between disease factors and hand bone loss in RA, but most of these studies have focused on early disease [7, 8, 9, 15, 22, 23]. The change in DXA-BMD hand and DXA bone mass content (BMC) has been found to be inversely correlated to elevated baseline CRP levels and the presence of RF [7, 8, 15]. However, loss of DXA-BMD and DXA-BMC has been suggested to occur only in patients with disease duration less than 2–3 years may limit the use of hand DXA-BMD in established disease [7-9]. Degenerative bone changes and increased inflammation in the small joints of the hand in the first years of the disease has been suggested partly to explain this finding [5, 7, 9]. The fact that DXR-BMD and DXA-BMD are based on different techniques and the precision for the DXR-method [18, 24, 25] is superior to the DXA method [26-28] may also contribute to this difference.

There are a few studies concerning disease activity as a predictor for DXR-BMD. In early RA both loss of DXR-BMD and DXR metacarpal cortical index (MCI) has been predicted by high CRP [22, 29], high ESR and anti-CCP [23, 30]. Only one previous study has shown that high DAS-28 predicted DXR-BMD loss in established RA [9]. However, this previous study only had follow-up of 2 years, but included a larger number of patients than the current study.

The DAS-28 levels were improved during the 5-year follow-up from 4.8 to 3.8 ($p<0.001$). The main reason for this is probably the introduction of anti-TNF therapy and a more aggressive treatment with combination of DMARDs.

Despite improvement of DAS-28 during the follow-up period, the patients experienced a significant decline in cortical hand BMD, suggesting that this bone loss is chronic and progressive.

Another observation in the present study was that the youngest age group lost more hand BMD than the oldest age group. In order to look at the possible impact of menopause on hand bone loss, we dichotomised the age into under and above 60 years, this also coincided with the median age. An earlier 10-year longitudinal study have suggested that the menopause, and not age per se was determining the start of a period with increased rate of bone loss [31]. However, some studies suggest that bone loss is increasing after menopause and then decline with age [32], while others suggest an increased constant bone loss [31]. This study might support that bone loss is larger in the first years after menopause [32], and suggest that not only increased generalised bone is observed in early postmenopausal women, but also that local hand bone loss is elevated. However, this result may be biased by the fact that the drop-outs were older than the participants (64 vs. 60 years, $p<0.001$).

The effect of medical treatment on bone was difficult to evaluate in this study due to the observational study design and the unstandardised patient treatment. In RA patients, adverse effects on bone by corticosteroids are of clinical importance [33, 34]. However, suppressing inflammation by corticosteroids has been found to increase hand DXR-BMD in two studies [22, 35], which may suggest that the anti-inflammatory effect overweighs the negative corticosteroid effect on bone. In the present study the use of corticosteroids was not associated with cortical bone loss, which might be due to the treatment with bone protecting agents. Use of bone protecting agents during follow-up was non-significantly associated with a decreased loss in DXR-BMD (Table 2) which may suggest that this treatment might not only have a beneficial effect on generalised bone loss and fractures but also on cortical hand bone loss in RA. Previous studies are conflicting in their results as to the effect of anti-resorptive treatment on hand BMD [22, 36, 37]. Anti-TNFα therapy has also been found to decrease bone loss in early RA patients [22, 38]. In this study treatment with anti-TNFα therapy was not associated with DXR-BMD change, which might be explained by the long disease duration, high disease activity and the low proportion (19%) of users of anti-TNFα therapy as well as confounding by indication.

The main limitation of this study was that the 5-year follow-up radiographs were analysed in three different ways. This was due to difficulties of analysing DXR-BMD in the three different centres as mentioned under the method section. However, DXR-BMD change did not differ between the three centres and the 5-year DXR-BMD median percentage changes for all centres were -6.7% which exceeds the possible source of error for the analysing methods. Based on these observations, we do not think the bias of this different analyses affect the main conclusion. Another limitation to the DXR method is that it can not be applied on radiographs of RA patients with severe joint damage. In the present study 10 and 7% of the baseline and follow-up radiographs could not be analysed for this reason.

Conclusion

In this study we found that RA patients with established disease experienced substantial cortical hand bone loss. This bone loss was independently predicted by high disease activity.

This finding indicates that increased disease activity leads to localised bone loss in longstanding RA which supports the importance of tight control and aggressive antiinflammatory treatment also in patients with established disease.

REFERENCES

1 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

2. Pettit AR, Ji H, von Stechow D, Müller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001; 159:1689-99.

3. Romas E, Gillespie MT. Inflammation-induced bone loss: can it be prevented? Rheum Dis Clin North Am 2006; 32:759-73.

4. Schett G. Cells of the synovium in rheumatoid arthritis. Osteoclasts. Arthritis Res Ther 2007; 9:203.

5. Deodhar AA, Brabyn J, Jones PW, Davis MJ, Woolf AD. Measurement of hand bone mineral content by dual energy X-ray absorptiometry: development of the method, and its application in normal volunteers and in patients with rheumatoid arthritis. Ann Rheum Dis 1994; 53:685-90.

6. Rosholm A, Hyldstrup L, Backsgaard L, Grunkin M, Thodberg HH. Estimation of bone mineral density by digital X-ray radiogrammetry: theoretical background and clinical testing. Osteoporos Int 2001; 12:961-9.

7. Deodhar AA, Brabyn J, Pande I, Scott DL, Woolf AD. Hand bone densitometry in rheumatoid arthritis, a five year longitudinal study: an outcome measure and a prognostic marker. Ann Rheum Dis 2003; 62:767-70.

8. Deodhar AA, Brabyn J, Jones PW, Davis MJ, Woolf AD. Longitudinal study of hand bone densitometry in rheumatoid arthritis. Arthritis Rheum 1995; 38:1204-10.

9. Hoff M, Haugeberg G, Kvien TK. Hand bone loss as an outcome measure in established rheumatoid arthritis: 2-year observational study comparing cortical and total bone loss. Arthritis Res Ther 2007; 9:R81.

10. Wolfe F, Sharp JT. Radiographic outcome of recent-onset rheumatoid arthritis: a 19-year study of radiographic progression. Arthritis Rheum 1998; 41:1571-82.

11. Lodder MC, Haugeberg G, Lems WF, Uhlig T, Orstavik RE, Kostense PJ, Dijkmans BA, Kvien TK, Woolf AD; Oslo-Truro-Amsterdam (OSTRA) Collaborative Study. Radiographic damage associated with low bone mineral density and vertebral deformities in rheumatoid arthritis: the Oslo-Truro-Amsterdam (OSTRA) collaborative study. Arthritis Rheum 2003; 49:209-15.

12. Haugeberg G, Lodder MC, Lems WF, Uhlig T, Ørstavik RE, Dijkmans BA, Kvien TK, Woolf AD. Hand cortical bone mass and its associations with radiographic joint damage and fractures in 50-70 year old female patients with rheumatoid arthritis: cross sectional Oslo-Truro-Amsterdam (OSTRA) collaborative study. Ann Rheum Dis 2004; 63:1331-4.
13. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44-8.
14. Barnett E, Nordin BE. The radiological diagnosis of osteoporosis: a new approach. Clin Radiol 1960; 11:166-74.
15. Haugeberg G, Green MJ, Quinn MA, Marzo-Ortega H, Proudman S, Karim Z, Wakefield RJ, Conaghan PG, Stewart S, Emery P. Hand bone loss in early undifferentiated arthritis: evaluating bone mineral density loss before the development of rheumatoid arthritis. Ann Rheum Dis 2006; 65:736-40.
16. Malich A, Boettcher J, Pfeil A, Sauner D, Heyne JP, Petrovitch A, Hansch A, Linss W, Kaiser WA. The impact of technical conditions of X-ray imaging on reproducibility and precision of digital computer-assisted X-ray radiogrammetry (DXR). Skeletal Radiol 2004; 33:698-703.
17. Böttcher J, Pfeil A, Rosholm A, Malich A, Petrovitch A, Heinrich B, Lehmann G, Mentzel HJ, Hein G, Linss W, Kaiser WA. Influence of image-capturing parameters on digital X-ray radiogrammetry. J Clin Densitom 2005; 8:87-94.
18. Hoff M, Dhainaut A, Kvien TK, Forslind K, Kalvesten J, Haugeberg G. Short-time in vitro and in vivo precision of direct digital X-ray radiogrammetry. J Clin Densitom 2009; 12:17-21.
19. van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol 2000; 27:261-3.
20. Landewe R, van der Heijde D. Radiographic progression depicted by probability plots: presenting data with optimal use of individual values. Arthritis Rheum 2004; 50:699-706.
21. van Gestel AM, Anderson JJ, van Riel PL, Boers M, Haagsma CJ, Rich B, Wells G, Lange ML, Felson DT. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. J Rheumatol 1999; 26:705-11.
22. Güler-Yüksel M, Allaart CF, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, van Groenendael JH, Mallée C, de Bois MH, Breedveld FC, Dijkmans BA, Lems WF. Changes in hand and generalised bone mineral density in patients with recent-onset rheumatoid arthritis. Ann Rheum Dis 2009; 68:330-6.
23. Bøyesen P, Hoff M, Odegård S, Haugeberg G, Syversen SW, Gaarder PI, Okkenhaug C, Kvien TK. Antibodies to cyclic citrullinated protein and erythrocyte sedimentation rate predict hand bone loss in patients with rheumatoid arthritis of short duration: a longitudinal study. Arthritis Res Ther 2009; 11:R103.
24. Jørgensen JT, Andersen PB, Rosholm A, Bjarnason NH. Digital X-ray radiogrammetry: a new appendicular bone densitometric method with high precision. Clin Physiol 2000; 20:330-5.
25. Hoff M, Dhainaut A, Kvien TK, Haugeberg G. Short time precision assessed with digital X-ray radiogrammetry in healthy individuals and rheumatoid arthritis patients. Ann Rheum Dis 2008; 67(suppl 2):563.

26. Alenfeld FE, Diessel E, Brezger M, Sieper J, Felsenberg D, Braun J. Detailed analyses of periarticular osteoporosis in rheumatoid arthritis. Osteoporos Int 2000; 11:400-7.
27. Daragon A, Krzanowska K, Vittecoq O, Ménard JF, Hau I, Jouen-Beades F, Lesage C, Bertho JM, Tron F, Le Loët X. Prospective X-ray densitometry and ultrasonography study of the hand bones of patients with rheumatoid arthritis of recent onset. Joint Bone Spine 2001; 68:34-42.
28. Haugeberg G, Green MJ, Conaghan PG, Quinn M, Wakefield R, Proudman SM, Stewart S, Hensor E, Emery P. Hand bone densitometry: a more sensitive standard for the assessment of early bone damage in rheumatoid arthritis. Ann Rheum Dis 2007; 66:1513-7.
29. Hoff M, Kvien TK, Elden A, Kalvesten J, Kavanaugh AF, Haugeberg G. Adalimumab reduces hand bone loss in rheumatoid arthritis independent of clinical response: Subanalysis of the PREMIER study. Ann Rheum Dis 2009; 68(suppl 3):452.
30. Geusens PP, Lems WF. Measuring metacarpal cortical bone by digital X-ray radiogrammetry: a step forward? Arthritis Res Ther 2009; 11:127.
31. Falch JA, Sandvik L. Perimenopausal appendicular bone loss: a 10-year prospective study. Bone 1990; 11:425-8.
32. Hui SL, Wiske PS, Norton JA, Johnston CC Jr. A prospective study of change in bone mass with age in postmenopausal women. J Chronic Dis 1982; 35:715-25.
33. Woolf AD. An update on glucocorticoid-induced osteoporosis. Curr Opin Rheumatol 2007; 19:370-5.
34. Lems WF. Bisphosphonates and glucocorticoids: effects on bone quality. Arthritis Rheum 2007; 56:3518-22.
35. Haugeberg G, Strand A, Kvien TK, Kirwan. JR Reduced loss of hand bone density with prednisolone in early rheumatoid arthritis: results from a randomized placebo-controlled trial. Arch Intern Med 2005; 165:1293-7.
36. Hyldstrup L, Jørgensen JT, Sørensen TK, Baeksgaard L. Response of cortical bone to antiresorptive treatment. Calcif Tissue Int 2001; 68:135-9.
37. Goldring SR. Periarticular bone changes in rheumatoid arthritis: pathophysiological implications and clinical utility. Ann Rheum Dis 20090; 68:297-9.
38. Hoff M, Kvien TK, Kälvesten J, Elden A, Haugeberg G. Adalimumab therapy reduces hand bone loss in early rheumatoid arthritis: explorative analyses from the PREMIER study. Ann Rheum Dis 2009; 68:1171-6.

Chapter 4a

The short-term effects of infliximab on the lipid profile in patients with rheumatoid arthritis

M. Vis
M.T. Nurmohamed
G.J. Wolbink
A.E. Voskuyl
M.H.M.T. de Koning
R.J. van der Stadt
J. Twisk
B.A.C. Dijkmans
W.F. Lems

J Rheumatol 2005 Feb; 32(2):252-5

ABSTRACT

Background: Cardiovascular morbidity and mortality appear to be enhanced in rheumatoid arthritis (RA) which might be due to an increased prevalence of cardiovascular risk factors such as dyslipidemia. It was recently shown that effective DMARD treatment had a favourable influence on the lipid profile in active RA. As infliximab markedly reduces disease activity in RA, the effects of infliximab on the lipid profile were examined.

Objective: To investigate the effect of infliximab on the lipid profile in patients with active RA.

Methods: Patients with active RA (defined as a Disease Activity Index 28 score ((DAS-28) of at least 3.2) were included. Infliximab (3mg/kg) was administered at baseline, 2 and 6 weeks. Total cholesterol and HDL-cholesterol levels were measured and their ratio, the atherogenic index, which is an important cardiovascular risk factor, calculated to assess changes in lipid profiles.

Results: Sixty-nine patients were enrolled and DAS-28 (SD) was 5.9 (± 1.4) at baseline and decreased to 4.6 (± 1.4) after 2 weeks and further to 4.1 (± 1.5) after 6 weeks. Total cholesterol level was 5.2 mmol/l at baseline and increased to 5.7 mmol/l ($p<0.001$) at 2 weeks and was 5.6 mmol/l ($p<0.001$ vs baseline) at week 6. For HDL-cholesterol these values were 1.5, 1.6 ($p<0.001$) and 1.6 mmol/l ($p<0.001$ vs baseline), respectively. Linear regression showed that changes in disease activity (DAS-28) were significantly inversely associated with changes in total cholesterol and HDL-cholesterol levels. The atherogenic index however remained constant during these six weeks of infliximab treatment. Corticosteroid use at baseline was associated with a significantly higher total cholesterol and HDL-cholesterol levels and a lower more favourable atherogenic index at baseline.

Conclusions: Treatment with infliximab induced a significant increase in levels of both total cholesterol and HDL-cholesterol, which was correlated with a decrease in disease activity. However this was not accompanied by a favourable effect on the atherogenic index.

INTRODUCTION

Mortality appears to be increased in patients with rheumatoid arthritis (RA) compared to the general population, and cardiovascular disease is the most important cause of death [1]. Theoretically, this increased cardiovascular risk in RA patients could be caused by (1) an increased prevalence of classical risk factors for cardiovascular disease such as dyslipidemia, diabetes mellitus, hypertension, body mass index, physical fitness and smoking habits (2) RA itself by either a) the underlying inflammatory process, or b) decreased functional capacity c) DMARD therapy, and (3) under-treatment of cardiovascular co-morbidity. This increased cardiovascular risk is also evidenced by an increased prevalence of sub-clinical atherosclerosis as assessed by increased carotid intima-media thickness in patients with RA in comparison with healthy controls [2, 3].

We previously demonstrated that active RA is associated with an unfavourable lipid profile, i.e. decreased total cholesterol and relatively more depressed HDL-cholesterol levels in comparison with age and sex-matched RA patients in remission [4]. This so-called atherogenic index i.e. the ratio between total cholesterol and HDL-cholesterol, which is an important cardiovascular risk factor, tended to normalise upon anti-rheumatic treatment. This normalisation occurred more rapidly in combination treatment with methotrexate, sulphasalazine and corticosteroids as compared with treatment with sulphasalazine alone [4]. Ultimately, these favourable alterations of the lipid profile could result in a lower cardiovascular risk. The beneficial effects of antirheumatic treatment in the above mentioned investigation could be mediated either through a direct effect of corticosteroids or indirect by influencing the disease activity.

Literature regarding the effect of treatment with anti-TNF blocking agents on the lipid profile in RA patients with active disease is scarce. Only one group investigated the effect of anti-TNF in a very limited number RA and psoriatic arthritis patients. They found that the lipid profile changed into a more atherogenic profile during treatment with infliximab [5].

Therefore, we prospectively investigated the effects of the anti-tumor-necrosis-factor (TNF) infliximab, a drug which has no known direct effects on the lipid profile, in large cohort of patients with active RA.

PATIENTS AND METHODS

Consecutive patients with active RA (defined as a Disease Activity Index 28 Joint Score (DAS-28, [6]) of at least 3.2) who were referred to the Slotervaart hospital for treatment with infliximab, were included. All patients fulfilled the ACR 1987 criteria of RA [7]. Infliximab (3mg/kg) was administered at 0, 2 and 6 weeks. All blood samples (non-fasting) were collected, in the morning, prior to each infusion and stored at -70 °C for a maximum of 6 months until lipid determinations. Total cholesterol, and HDL-cholesterol levels were measured and their ratio calculated to assess changes in lipid profiles.

At each visit erythrocyte sedimentation rate, C-reactive protein, Visual Analogue Scale of general health, number of swollen and tender joints were assessed in order to determine disease activity (DAS-28 score). Changes in medication were recorded at each visit.

Total cholesterol and HDL-cholesterol determinations

Total serum cholesterol (N: 5.0–6.4 mmol/l) was measured by an enzymatic method in an autoanalyser; HDL-cholesterol (N > 0.9 mmol/l) was determined enzymatically with PEG-modified enzymes.

Although, the classical (chemical) reference value considers a total cholesterol value of 6.4 mmol/l as the upper limit of normal we have, for the interpretation of our results, chosen 5.0 mmol/l as the upper limit in view of the recommendations made by several national and international consensus committees [8, 9]. These guidelines were made in view of the increasing evidence that "high-normal" total cholesterol serum levels already imply an increased cardiovascular risk.

Statistical analysis

Total cholesterol, HDL- and the total cholesterol / HDL-ratio (atherogenic index) at 2 and 6 weeks were normally distributed and therefore compared to baseline with paired t-tests.

To investigate the association between the changes in lipid profiles (outcome variables) and changes in disease activity, linear regression analyses were performed for the period from 0 to 2 weeks and the period from 0 to 6 weeks. Two analyses were done: one crude without adjustments and one adjusted for age, disease duration, sex and change in prednison dose. All analyses were carried out with SPSS (version 11.0).

RESULTS

Patients

A total of 69 consecutive patients were enrolled, with the following baseline characteristics (Table 1): 55 (80%) female, mean age 55 (13) years, mean disease duration 12 years (range 0–59 years) and 48 (70%) rheumatoid factor positive. At entry, 90% of patients used methotrexate (MTX) (mean dose 15.8 mg/week) and five patients used other DMARDs (Table1).

Thirty-two patients used corticosteroids at the start of the study (mean prednisone dose 10.6 mg/day). In the patients using prednisone; 19 patients kept a stable dose whereas in 13 patients the dose was decreased because of a good response to infliximab. DAS-28 score was 5.9 (1.4) at baseline and decreased to 4.6 (1.4) after 2 weeks ($p<0.001$) and further to 4.1 (1.5) after 6 weeks ($p<0.001$) (Table 2).

Total cholesterol and HDL-cholesterol

Total cholesterol level and HDL-cholesterol were was 5.17 mmol/l and 1.47 mmol/l at baseline respectively. Total cholesterol and HDL-cholesterol levels were significantly higher at baseline in the steroid user group than in the patients without steroid

Table 1 Baseline characteristics of 69 RA patients

Baseline characteristics	
Age (years)	
Mean (SD)	55 (13)
Range	24–80
Female, n (%)	55 (80)
Rheumatoid factor postive, n (%)	48 (70)
Mean duration of disease, years (range)	12 (0–59)
DAS-28 at baseline, mean (SD)	5.9 (1.4)
Treatments	
Steroids, n (%)	32 (46)
Mean dose, mg (range)	10.6 (2.5–30)
Methotrexate, n	62 (90)
Mean dose, mg (range)	10.8 (2.5–30)
Other DMARDs, n	
D-penicillamine	1
Azathioprine	3
Cyclosporine	1
Hydroxychloroquine	5

Table 2 DAS-28, ESR, total cholesterol, HDL-cholesterol and atherogenic index (cholesterol/HDL-cholesterol) levels during the 6-week treatment

	Baseline	2 weeks	6 weeks
DAS-28	5.9 (1.4)	4.9* (1.4)	4.1* (1.5)
Total cholesterol mmol/l (SD)	5.17 (1.06)	5.7* (1.17)	5.52* (1.24)
HDL-cholesterol mmol/l (SD)	1.47 (0.43)	1.60* (0.47)	1.59* (0.50)
Atherogenic index	3.8 (1.2)	3.7 (1.1)	3.7 (1.2)

* p<0.001 compared to baseline.

treatment (respectively 5.52 vs. 4.86 p<0.001 and 1.72 vs 1.25 p<0.001). This resulted in a more favourable atherogenic index in the steroid user group, at baseline (respectively 3.41 vs. 4.10, p<0.05). Age, gender, and disease activity were not significantly different between these groups.

Total cholesterol increased from 5.17 mmol/l to 5.70 mmol/l (p<0.001 vs. baseline) at 2 weeks and was 5.52 mmol/l (p<0.001 vs. baseline) at week 6. The values for HDL-cholesterol were 1.47, 1.60 (p<0.001 vs. baseline) and 1.59 mmol/l (p<0.001 vs. baseline) respectively. The atherogenic index however remained constant during the six weeks of infliximab treatment (Table 2).

To assess whether changes in the lipid profile were associated with a change in disease activity we performed linear regression analyses. The changes in HDL- and total cholesterol from 0 to 2 weeks showed no significant association with changes in DAS-28. However, changes in DAS-28 from 0 to 6 weeks were significantly inversely associated with changes in both total- and HDL-cholesterol levels (Table 3). This association remained after adjusting for changes in prednisone dose, age, sex and disease duration. Although the mean atherogenic index did not change, changes in DAS-28 were significantly associated with changes in the atherogenic index in the period from 0 to 2 weeks.

However, this association disappeared when considering the whole study period (from 0 to 6 weeks) (Table 3).

Because prednisone had a significant confounding effect in our model, we performed the same regression analyses for the change in prednisone. Changes in prednisone dose were significantly associated with the changes in HDL and inversely associated with the atherogenic index from 0 to 6 weeks (respectively B= 0.031, p=0.005 and B=0.0453, p=0.047). This association remained after adjusting for the change in disease activity. No other relations between changes in prednisone and lipid profile were found at either of the intervals.

Table 3 Results of the linear regression models to evaluate the association between changes in lipid profile and changes in DAS-28 from 0 to 2 weeks and 0 to 6 weeks

	Coefficient	Significance	95% CI	
	B	p	Lower	Upper
Changes HDL from 0 to 2 weeks				
Changes in DAS-28	-0.04	0.13	-0.083	0.011
Corrected	-0.04	0.14	-0.084	0.012
Changes HDL from 0 to 6 weeks				
Changes in DAS-28	-0.06	0.01	-0.099	-0.014
Corrected	-0.05	0.01	-0.093	-0.012
Changes total cholesterol from 0 to 2 weeks				
Changes in DAS-28	0.02	0.77	-0.125	0.168
Corrected	0.01	0.88	-0.1	0.157
Changes total cholesterol from 0 to 6 weeks				
Changes in DAS-28	-0.15	0.02	-0.27	-0.025
Corrected	-0.16	0.01	-0.28	-0.042
Changes atherogenic index from 0 to 2 weeks				
Changes in DAS-28	0.14	0.01	0.04	0.245
Corrected	0.13	0.02	0.03	0.238
Changes atherogenic index from 0 to 6 weeks				
Changes in DAS-28	0.05	0.23	-0.03	0.140
Corrected	0.04	0.41	-0.05	0.121

The corrected model gives the results of the studied relation after correction for age, sex, disease duration and changes in prednisone dosage.

DISCUSSION

We found slightly elevated total cholesterol levels and normal HDL-cholesterol levels in RA patients with active disease at baseline. Treatment with infliximab induced a significant increase in levels of both total cholesterol and HDL-cholesterol. However, these changes did not alter the atherogenic index which is an important prognostic marker for future cardiovascular disease. The increases in cholesterol levels were significantly associated with a decrease in disease activity.

The stable atherogenic index over the studied period contrasts with findings in early RA-cohorts [4, 10]. One trial, investigating combination DMARD therapy (including corticoids), indicated a favourable effect on the atherogenic index. However, this study was performed in early RA patients. The explanation could be that rheumatoid cachexia [11], with accompanying low cholesterol levels and relatively lower HDL-cholesterol levels, is more prominent in early RA than in established RA. The other study demonstrated decreasing HDL-cholesterol levels during treatment with

infliximab leading to a less favourable atherogenic index [4]. However, in this latter study only seven RA and eight psoriatic arthritis patients were studied, hence a chance finding cannot be excluded.

Thus far the literature is contradictory regarding the effect of corticosteroids on lipid profile in RA and data of properly designed studies are lacking. The results of this study indicate that use of corticosteroids was, at baseline, associated with increased total cholesterol levels and, to a relatively greater extent, increased HDL-cholesterol levels. Consequently, a lower atherogenic index, in prednisone users in comparison to non-prednisone users, was observed at baseline. Lowering the prednisone dose was associated with a decreasing HDL-cholesterol level, and an increase of the atherogenic index. Whether or not this favourable effect of corticosteroids on the lipid profile, at baseline, is ultimately offset by other cardiovascular side effects of corticosteroid use, e.g. hypertension or hyperglycemia, remains to be determined. The effect of lowering prednisone attenuates the effect of a decreasing disease activity (by infliximab) on the atherogenic index and could explain the observation that the atherogenic index did not change during the studied period.

Altogether, this study shows that short-term treatment with infliximab increases both total cholesterol and HDL-cholesterol and that this was associated with a decrease of disease activity. However, the atherogenic index remained constant due to lowering the prednisone doses.

Hence, the favourable effect of infliximab on cardiovascular comorbidity observed in the literature appears not to be mediated by effects on the lipid metabolism [12].

REFERENCES

1 Van Doornum, McGoll, Wicks IP. Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis. Arthritis Rheum 2002; 46:862-73.

2 Park YB, Ahn CW, Choi HK, Lee SH, In BH, Lee HC, Nam CM, Lee SK. Atherosclerosis in rheumatoid arthritis: morphologic evidence obtained by carotid ultrasound. Arthritis Rheum 2002; 46:1714-9.

3 Kumeda Y, Inaba M, Goto H, Nagata M, Henmi Y, Furumitsu Y, Ishimura E, Inui K, Yutani Y, Miki T, Shoji T, Nishizawa Y. Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. Arthritis Rheum 2002; 46:1489-97.

4 Boers M, Nurmohamed MT, Doelman CJ, Lard LR, Verhoeven AC, Voskuyl AE, Huizinga TW, van der Stadt RJ, Dijkmans BA, van der Linden S. The influence of glucocorticoids and disease activity on total and HDL-cholesterol in patients with rheumatoid arthritis (RA). Ann Rheum Dis 2003; 62:842-5.

5 Cauza E, Cauza K, Hanusch-Enserer U, Etemad M, Dunky A, Kostner K. Intravenous anti-TNF-alpha therapy leads to elevated triglyceride and reduced HDL-cholesterol levels in patients with rheumatoid and psoriatic arthritis. Wien Klin Wochenschr 2002; 30:1004-7.

6 Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL Modified disease activity index that include twenty-eight joint counts. Arthritis Rheum 1995; 38:44-8.

7 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

8 Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285:2486-97.

9 Health Council of the Netherlands. Cholesterol-lowering therapy. The Hague: Health Council of the Netherlands, 2000; publication no. 2000/17.

10 Park YB, Choi HK, Kim MY, Lee WK, Song J, Kim DK, Lee SK. Effects of antirheumatic therapy on serum lipid levels in patients with rheumatoid arthritis: a prospective study. Am J Med 2002; 113:188-93.

11 Walsmith J, Roubenoff R. Cachexia in rheumatoid arthritis. Int J Cardiol 2002; 85:89-99.

12 Jacobson LTH, Turesson C, Gülfe A, Crnkic M, Peterson IF, Saxne T, et al. Low incidence of first cardiovascular event in rheumatoid arthritis patients treated with TNF-blockers Arthritis Rheum 2003; 48(suppl): S241.

Chapter 4b

Changes in lipid profile during infliximab treatment and corticosteroid therapy in rheumatoid arthritis

M.J.L. Peters
M. Vis
V.P. van Halm
G.J. Wolbink
A.E. Voskuyl
W.F. Lems
B.A.C. Dijkmans
J.W.R. Twisk
M.H.M.T. de Koning
R.J. van de Stadt
M.T. Nurmohamed

Ann Rheum Dis 2007 Jul; 66(7):958-61

ABSTRACT

Objectives: To evaluate the effects of infliximab and corticosteroid therapy on the lipid profile in patients with active rheumatoid arthritis (RA).

Methods: Infliximab infusions were given at week 0, 2, 6 and then every 8 weeks. Prior to each infusion, disease activity parameters (DAS-28, CRP) and lipid levels (total-, high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein A-1 (Apo A-1) and apolipoprotein B) were measured in 80 consecutive RA patients, who completed the study period of 48 weeks. Longitudinal analyses were used to investigate (1) the course of lipid levels over a period of time and (2) the relationship between lipids, prednisone dose and disease activity.

Results: Infliximab treatment causes a significant reduction in disease activity and a concomitant decrease of prednisone dose. Although they initially improved significantly, all lipid levels had returned to baseline levels after 48 weeks, except for Apo A-1. Longitudinal analyses reveal 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 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.

Conclusion: Overall, no changes in serum lipid levels were observed after 48 weeks of infliximab treatment. The initial beneficial effects of infliximab on the lipid profile, by means of a reduction of disease activity, are attenuated by a concomitant decrease in prednisone dose.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory disorder of the joints. Patients with RA have an increased morbidity and mortality compared to the general population. Mortality due to cardiovascular disease (CVD) is the leading cause of death observed in patients with RA [1]. This excess cardiovascular mortality in patients with RA is predominantly due to accelerated atherosclerosis [2].

A well-known cause of atherosclerosis is an atherogenic lipid profile. In particular, low levels of high-density lipoprotein (HDL) cholesterol, and high levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides are associated with an increased prevalence of CVD in the general population [3]. An important prognostic indicator for future CVD is the atherogenic index, i.e. the ratio between total- and HDL-cholesterol [4]. New data indicate that apolipoproteins, which are protein parts of the lipoprotein complex, are possibly better predictors for CVD. High levels of apolipoprotein B (apo B) and an increased apolipoprotein B / apolipoprotein A-1 ratio (apo B / apo A-1) are associated with an increased cardiovascular risk, whereas higher levels of apolipoprotein A-1 (apo A-1) are protective for developing CVD [5, 6].

Several studies reported that RA is associated with an unfavorable lipid profile, particularly, in patients with active disease [7-9]. These studies show that active RA is associated with lower levels of HDL-cholesterol compared to healthy controls leading to a higher, i.e. unfavorable, atherogenic index. Hence, an atherogenic lipid profile may be part of the cause of the increased cardiovascular risk in patients with RA.

There is growing evidence that inflammation in RA is associated with a worsening of the lipid profile [10, 11], which improves upon effective anti-rheumatic treatment [12, 13]. We previously reported that normalization of the lipid profile in patients with RA occurred more rapidly in combination treatment with methotrexate (MTX), sulfasalazine, and prednisone than in treatment with sulfasalazine alone, thereby demonstrating the temporal relationship between decreasing disease activity and improvement of the atherogenic index [14]. However, due to the limited duration of the study we were unable to answer the question whether this difference was due to improved disease suppression or changes in prednisone dose.

In another study, we found that treatment with anti-tumour necrosis factor (aTNF) therapy leads to a significant increase of both total- and HDL-cholesterol levels, which was inversely related with disease activity. The atherogenic index, however, remained constant during the six weeks study period [15].

Thus far, data regarding lipid changes during immunosuppressive treatment are contradictory [16, 17] and studies observing lipid changes during long-term

immunosuppressive treatment are lacking. In the present study longitudinal analyses are performed to investigate (1) the longitudinal course of lipid levels, including apolipoproteins, over time during immunosuppressive treatment, i.e. infliximab, and (2) the longitudinal relationship between lipids and indicators of disease activity and between lipids and prednisone doses.

PATIENTS AND METHODS

Patients

Consecutive patients with active RA (defined as a Disease Activity Index 28 Joint Score (DAS-28) of at least 3.2) who were referred to the Slotervaart Hospital for treatment with infliximab were included. All RA patients fulfilled the American College of Rheumatology criteria [18]. Infusions with infliximab were given at weeks 0, 2, 6 and 14 weeks and from thereon every 8 weeks. Generally, infliximab was given in combination with stable doses of methotrexate. Infliximab was administered intravenously in a starting dose of 3 mg/kg. In patients with inadequate response as judged by the patients' rheumatologist the dose of infliximab could be increased to 7.5 mg/kg. Blood samples (nonfasting) were collected in the morning, prior to each infusion on week 0, 6, 22 and 48 and were stored at -70 °C until analyses. Total cholesterol, HDL-cholesterol, triglycerides, apo A-1 and apo B concentrations were measured and the total / HDL-cholesterol and apo B / apo A-1 ratios were calculated. At each visit disease activity, defined as DAS-28 [19] and drug treatment was assessed. In total, 108 patients were included in the cohort, but 28 patients dropped out in the first year of treatment: 22 because of non-response (78.5%), 5 because of side effects (18%) and 1 patient (3.5%) who died owing to infliximab-unrelated events. Thus, the data from 108 patients were eligible for analyses. The baseline characteristics of the dropout patients were comparable to those of the patients included in the study (data not shown). Thus, all included patients completed the study period of 48 weeks and the patients who dropped out (n=28) were not included for the analysis. There were no (additional) exclusion criteria.

Lipids

Total serum cholesterol (N < 5.0 mmol/l (193 mg/dl)) and triglycerides (N < 2.2 mmol/l, 196 mg/dl)) were measured by an enzymatic method using Roche clinical chemistry analyzers. HDL-cholesterol (N men > 0.9 mmol/l (35 mg/dl), women > 1.1 mmol/l (42 mg/dl)) was determined enzymatically with PEG-modified enzymes. The atherogenic index was calculated using the following formula: atherogenic index = total

/ HDL-cholesterol. Apo A-1 (N men 1.04-2.02 g/l, women 1.08-2.25 g/l) and apo B (N men 0.66-1.33 g/l, women 0.60-1.17 g/l) were analyzed by an immunoturbidimetric method, using assays supplied by Roche Diagnostics.

As it was expected that only small differences in the concentration of analyses could be significant, collected sera were first stored frozen and subsequently analyzed on the same day in a single run with all sera belonging to one person grouped together. This set-up produces within group analytical coefficients of variation for the analyses in their reference range of less than 1%.

Statistical analyses

As some patients were treated with prednisone, differences in lipid profiles between prednisone users (yes/no) were analyzed by using independent t-tests for normally distributed variables (i.e. HDL-cholesterol, apo A-1, apo B, and the apo B / apo A-1 ratio), and Mann Whitney U tests were used for not normally distributed variables (i.e. total cholesterol, atherogenic index and triglycerides).

The time course of lipid levels during treatment with infliximab was investigated by using general estimation equations (GEEs). This regression technique is used as it adjust for dependency of several measurements within one subject and is capable of dealing with unequally spaced time intervals and with missing data [20]. For these analyses each lipid value was included separately as dependent variable and time was included as a categorical independent variable.

GEE analyses were also used to investigate the influence of indicators of disease activity (i.e. DAS-28 score and CRP) and prednisone dose on all lipid levels. For this we performed two analyses: (1) GEE analyses including a lipid value and an indicator of disease activity or prednisone dose; for example total cholesterol as dependent variable and DAS-28 as independent variable, total cholesterol as dependent variable and CRP as independent variable and total cholesterol as dependent variable and prednisone as independent variable; (2) GEE analyses including a lipid value and an indicator of disease activity corrected for prednisone dose. With GEE analyses the whole development of a certain lipid is associated with the development of disease activity or prednisone dose resulting in one regression coefficient. All analyses were adjusted for age and gender. As the distribution of total cholesterol, atherogenic index and triglycerides were not normal, a log transformation was performed before the GEE analyses. All analyses were performed with STATA (version 7) and p-values of less than 0.05 were considered statistically significant [21].

RESULTS

Patients

The study population consisted of 80 (62 women and 18 men) consecutively included RA patients with a mean age of 56 years (SD 14 years). The median disease duration was 10 years (range: 0–59 years), 55 patients (69%) were IgM-Rheumatoid factor positive and 67 patients (82%) had erosions. At baseline statins were used by 4 patients and 2 patients (2%) reported diabetes mellitus. During the study period none of the included patients started statins or glucose lowering therapy. Seventy-seven patients (96%) used concomitant methotrexate in a stable dose, i.e. 15 mg/wk at all time points and 35 patients (44%) used prednisone at the start of the study (mean prednisone dose 8.3 mg/day). As expected treatment with infliximab leads to a significantly better DAS-28-score at 48 weeks compared to baseline: 5.7 vs 3.9 ($p<0.01$).

Lipids

The course of lipid levels over time during treatment with infliximab is shown in Table 1. Patients who used prednisone had higher total- and HDL-cholesterol levels and a lower atherogenic index at baseline, but these differences did not reach statistical significance.

After six weeks infliximab treatment total cholesterol levels increased by 6.1% ($p<0.01$) and HDL-cholesterol levels increased by 10.3% ($p<0.01$), resulting in a 6.4% decrease of the atherogenic index ($p<0.01$). In the next period (6 to 22 weeks) both total- and

Table 1 Total cholesterol, HDL-cholesterol, triglycerides (mmol/l), Apo A-1, Apo B (g/l) and the atherogenic index and apo B/apo A-1 ratio during treatment with infliximab

Variable	Baseline	6 weeks	22 weeks	48 weeks
Total cholesterol (mmol/l), median (range)	4.79 (2.70–8.10)	5.08 (2.79–9.18)*	4.94 (2.90–10.42)*	4.81 (3.21–7.29)
HDL-cholesterol (mmol/l), mean (SD)	1.46 (0.50)	1.61 (0.54)*	1.55 (0.53)*	1.53 (0.51)
Atherogenic Index, median (range)	3.42 (1.74–7.78)	3.20 (1.59–8.63)*	3.43 (1.67–8.08)	3.38 (1.62–10.70)
Triglycerides (mmol/l), median (range)	1.26 (0.62–4.40)	1.39 (0.52–3.80)*	1.32 (0.46–3.36)	1.26 (0.62–3.48)
Apo A-1 (g/l), mean (SD)	1.58 (0.35)	1.70 (0.33)*	1.65 (0.33)*	1.65 (0.33)*
Apo B (g/l), mean (SD)	0.93 (0.23)	0.95 (0.24)	0.94 (0.24)	0.93 (0.19)
Apo B / Apo A-1 ratio (SD)	0.62 (0.20)	0.58 (0.20)*	0.59 (0.18)	0.59 (0.16)

* $p<0.05$ compared to baseline (performed by GEE analyses).

HDL-cholesterol levels decreased, but remained significantly elevated compared to baseline. At 48 weeks, total- and HDL-cholesterol levels approached baseline levels. As total- and HDL-cholesterol levels moved towards baseline levels the atherogenic index revealed no significant difference compared to the baseline index at 22 and 48 weeks. Triglycerides levels did not change during the whole treatment period.

Compared to baseline apo A-1 levels increased 4.5% at 48 weeks ($p<0.01$), whereas apo B levels and the apo B / apo A-1 ratio did not change significantly at 48 weeks compared to baseline.

Lipids and disease activity

The course of lipids over time was compared to the course of disease activity after adjustment for age and gender. GEE analyses yielded a significant inverse association between disease activity and lipid levels (Table 2). Changes in disease activity (DAS-28) were significantly related with changes in HDL- as well as total cholesterol levels. This means that if disease activity according to DAS-28 decreases one point, total cholesterol will raise 0.016 mmol/l (0.618 mg/dl) and HDL-cholesterol will raise 0.045 mmol/l (1.737 mg/dl), resulting in a more favorable i.e. lower, atherogenic index. Similarly, the individual inflammation parameter CRP was significantly inversely associated with both HDL- and total cholesterol levels. Moreover, the analyses showed that disease activity was inversely related with apo A-1, resulting in a lower, i.e. more favorable, apo B / apoA-1 ratio.

Table 2 Relationship between lipid items, indicators of disease activity and prednisone

Variable	Atherogenic index Coefficient (SE)	Total cholesterol Coefficient (SE)	HDL-cholesterol Coefficient (SE)
Indicators of disease activity			
1-point decrease/difference of DAS-28	-0.016 (0.008)*	0.016 (0.005)*	0.045 (0.013)*
10-points decrease/difference of CRP	-0.026 (0.001)*	0.024 (0.001)*	0.067 (0.001)*
Reduction/difference of 1 mg prednisone	0.009 (0.004)*	-0.004 (0.002)*	-0.016 (0.006)*
Variable	**Apo B / Apo A-1 Coefficient (SE)**	**Apo B Coefficient (SE)**	**Apo A-1 Coefficient (SE)**
Indicators of disease activity			
1-point decrease/difference of DAS-28	-0.013 (0.005)*	0.004 (0.004)	0.036 (0.009)*
10-points decrease/difference of CRP	-0.014 (0.001)*	0.009 (0.001)	0.045 (0.001)*
Reduction/difference of 1 mg prednisone	0.005 (0.002)*	0.001 (0.002)	-0.009 (0.004)*

Regression coefficients and standard errors estimated by GEE analyses for each lipid item as a continuous outcome variable. Atherogenic index, Total cholesterol (mmol/l), HDL-cholesterol (mmol/l), Apo B / Apo A-1 ratio, Apo A-1 (g/l) and Apo B (g/l). All analyses were adjusted for age and gender. * $p<0.05$.

Lipids and prednisone

During treatment with infliximab lipid levels were compared to changes in prednisone dose at different time points after adjustment for age and gender. Changes in prednisone dose were related to changes in HDL- and total cholesterol (Table 2). Prednisone changes, however, had a greater influence on HDL- than on total cholesterol levels resulting in an inverse association between prednisone dose and the atherogenic index. In other words, a higher prednisone dose is associated with a lower, i.e. more favorable atherogenic index. Furthermore, the prednisone dose appeared to have a significant positive relation with apo A-1, leading to a significant inverse association with the apo B to apo A-1 ratio.

The reported associations between lipids and disease activity and between lipids and prednisone did not change when prednisone and disease activity parameters were both included as independent variables in the same regression analysis (data not shown).

Due to the favourable effect of infliximab on the DAS-28 score, the prednisone dose was gradually decreased from a median dose of 8.3 to 4.6 mg/day. Subgroup analysis revealed no significant differences with regard to cholesterol levels between RA patients with prednisone and without prednisone at all time points.

DISCUSSION

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. As a result improvement of the atherogenic index at six weeks was not observed anymore from 22 weeks onwards. Longitudinal data analyses, i.e. GEE analyses, revealed that lowering the prednisone dose attenuated the effects of infliximab on total cholesterol and HDL-cholesterol levels and its main protein part apo A-1, thereby abolishing the improvement of the atherogenic index as well as the apo B to apo A-1 ratio. Nevertheless, apo A-1 levels remained significantly elevated after 48 weeks of treatment. This latter might be important as there is accumulating epidemiological evidence indicating that high levels of apo A-1 are strongly related with a lower risk for future CVD [5, 6].

Recent research has shown that systemic inflammation plays a pivotal role in the development of atherosclerosis. Immune cells dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions and inflammation elicits CVD [22]. Hence, inflammation might be the key explaining the enhanced cardiovascular risk in RA patients [23]. Moreover, inflammation induces the acute phase response leading to lipid changes and alterations in lipoprotein metabolism.

Most of these changes, i.e. decreased levels of HDL-cholesterol and apo A-1, are pro-atherogenic and ultimately they may contribute to the increased cardiovascular risk in RA. In this study, a decreasing disease activity resulted in a lower, atherogenic index as a result of higher total cholesterol levels and even more pronounced higher HDL-levels. In addition, a decrease of disease activity led to a better apo B to apo A-1 ratio, due to significantly increased apo A-1 levels. These findings are in line with other studies reporting an inverse association between inflammatory markers (ESR and CRP) and HDL-cholesterol or its apo A-1 [12, 13].

Differences reported in this study are small and may therefore question their clinical relevance. Several studies, however, illuminate the importance of lipid changes even though the observed differences are small. The Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) as well as the Framingham Study for example show that a 0.026 mmol/l (1 mg/dl) increment of baseline HDL-cholesterol is associated with an approximately 3.5% risk reduction for coronary heart disease [24, 25]. Another study performed by Rubins et al. revealed that a 6% increase in HDL-cholesterol is associated with an absolute risk reduction of 4.4% of death from coronary heart disease or non-fatal infarction [26]. Although these findings cannot be directly extrapolated to our study, they demonstrate the clinical importance of only small lipid changes. Moreover several recent observations indicate that apo-B and apo A-1 may in fact be more powerful predictors of risk for CVD than the conventional lipid values [5, 6, 22, 27]. This is important and might be clinically relevant as this study indicates that apo-A1 remains significantly elevated during the whole treatment period.

Mechanisms underlying the decrease of HDL-cholesterol during inflammation are not firmly established. Our findings, however, suggest that alterations in prednisone dose are relevant. We showed that lowering the prednisone dose results in a decrease of total cholesterol levels, that is predominantly due to a decrease in HDL-cholesterol leading to a higher atherogenic index (as the effect on total cholesterol was less pronounced). Vice versa, increasing the prednisone dose might have favorable effects on the lipid profile. Nevertheless, it is highly uncertain whether or not this postulated beneficial effect is ultimately offset by other cardiovascular side effects of long-term prednisone use, as insulin resistance and hypertension.

In addition to a previous study performed by our study group [14], the present study reveals additional insights into the influence of prednisone and disease activity on the lipid profile as both have a significant, but opposing, and more pronounced influence on HDL-cholesterol than on total cholesterol.

Methotrexate may provide a substantial survival benefit, largely by reducing cardiovascular mortality [28]. Patients in our investigation were on stable MTX doses during the treatment period. Hence, we were not able to evaluate the effect of MTX on the lipid

profile. Our results, however, do indicate that suppression of inflammation leads to a more favourable lipid profile which may also be conceivable for methotrexate.

There might be (additional) explanations for the observed longitudinal lipid changes. First, exercise increases HDL-cholesterol in healthy individuals [29] and immunosuppressive therapy is associated with a substantial clinical improvement whereby RA patients will increase their physical activity. Second, RA patients with a high disease activity have higher levels of tumour necrosis factor, which is associated with rheumatoid cachexia [30]. Treatment with infliximab obviously leads to lower levels of the tumour necrosis factor, therefore possibly leading to a better dietary intake resulting in increased lipid levels. Finally, we have to consider the possibility that the observed lipid changes are due to a direct effect of biologicals through blocking TNF-alpha.

In conclusion, the present investigation shows that immunosuppressive therapy, with infliximab, might have beneficial effects on the increased cardiovascular risk in RA patients through several pathways: (1) due to its anti-inflammatory effects, (2) indirectly as a cause of lower prednisone doses, and (3) through, albeit small, anti-atherogenic effect on the lipid profile. Prospective cardiovascular outcome studies are needed to establish whether or not immune-suppressive therapy indeed results in a lower cardiovascular risk.

REFERENCES

1 Van Doornum S, McGoll G, Wicks IP. Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis. Arthritis Rheum 2002; 46:862-73.

2 Park YB, Ahn CW, Choi HK, Lee SH, In BH, Lee HC, Nam CM, Lee SK. Atherosclerosis in rheumatoid arthritis: morphologic evidence obtained by carotid ultrasound. Arthritis Rheum 2002; 46:1714-9.

3 Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986; 256:2835-8.

4 Kinosian B, Glick H, Garland G. Cholesterol and coronary heart disease: predicting risk by levels and ratios. Ann Intern Med 1994; 121:641-7.

5 Walldius G, Jungner I. Apolipoprotein B and Apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004; 255:188-205.

6 Luc G, Bard JM, Ferrières J, Evans A, Amouyel P, Arveiler D, Fruchart JC, Ducimetière P. Value of HDL cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I/A-II in prediction of coronary heart disease: the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction. Arterioscler Thromb Vasc Biol 2002; 22:1155-61.

7 Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH, Lee J. Lipid profiles in untreated patients with rheumatoid arthritis. J Rheumatol 1999; 26:1701-4.

8 Choi HK, Seeger JD. Lipid Profiles Among US Eldery with Untreated Rheumatois Arthrits – The Third National Health and Nutrition Examination Survey. J Rheumatol 2005; 32:2311-6.

9 Yoo WH. Dyslipoproteinemia in Patients with Active Rheumatoid Arthritis: Effects of Disease Activity, Sex, and Menopausal Status on Lipid Profiles. J Rheumatol 2004; 31:1746-53.

10 Lee YH, Choi SJ, Ji JD, Seo HS, Song GG. Lipoprotein(a) and lipids in relation to inflammation in rheumatoid arthritis. Clin Rheumatol 2000;19:324-5.

11 Dursunoğlu D, Evrengül H, Polat B, Tanriverdi H, Cobankara V, Kaftan A, Kiliç M. Lp(a) lipoprotein and lipids in patients with rheumatoid arthritis: serum levels and relationship to inflammation. Rheumatol Int 2005, 25:241-5.

12 Munro R, Morrison E, McDonald AG, Hunter JA, Madhok R, Capell HA. Effect of disease modifying agents on the lipid profiles of patients with rheumatoid arthritis. Ann Rheum Dis 1997; 56:374-7.

13 Park YB, Choi HK, Kim MY, Lee WK, Song J, Kim DK, Lee SK. Effects of antirheumatic therapy on serum lipid levels in patients with rheumatoid arthritis: a prospective study. Am J Med 2002; 113:188-193.

14 Boers M, Nurmohamed MT, Doelman CJ, Lard LR, Verhoeven AC, Voskuyl AE, Huizinga TW, van de Stadt RJ, Dijkmans BA, van der Linden S. The influence of glucocorticoids and disease activity on total and HDL-cholesterol in patients with rheumatoid arthritis. Ann Rheum Dis 2003; 62:842-5.

15 Vis M, Nurmohamed MT, Wolbink G, Voskuyl AE, de Koning M, van de Stadt R, Twisk JW, Dijkmans BA, Lems WF. Short term effects of infliximab on the lipid profile in patients with rheumatoid arthritis. J Rheumatol 2005; 32:252-5.

16 Popa C, Netea MG, Radstake T, Van der Meer JW, Stalenhoef AF, van Riel PL, Barerra P. Influence of anti-tumour necrosis factor therapy on cardiovascular risk factors in patients with active rheumatoid arthritis. Ann Rheum Dis 2005; 64:303-5.

17 Irace C, Mancuso G, Fiaschi E, Madia A, Sesti G, Gnasso A. Effect of anti TNFalpha on arterial diameter and wall shear stress and HDL cholesterol. Atherosclerosis 2004; 177:113-8.

18 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

19 Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44-8.

20 Twisk JWR. Longitudinal data analysis: A comparison between generalized estimating equations and random coefficient analysis. Eur J Epidemiol 2004; 19:769-76.

21 Hamilton LC. Statistics with Stata, updated version 7. Duxbury Press, Belmont CA, 2002.

22 Hansson GK. Inflammation, Atherosclerosis, and Coronary Artery Disease. N Engl J Med 2005; 352:1685-95.

23 Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation 2003; 108:2957-63.

24 Gordon DJ, Knoke J, Probstfield JL, Superko R, Tyroler HA. High-density lipoprotein cholesterol and coronary heart disease in hypercholesterolemic men: the Lipid Research Clinics Coronary Primary Prevention Trial. Circulation 1986; 74:1217-25.

25 The Framingham Study: Coronary risk handbook using HDL-cholesterol for persons over 50. Bethesda, National Heart, Lung and Blood Institute, 1978.

26 Rubins HB, Robins SJ, Collins D. The Veterans Affairs High-Density Lipoprotein Intervention Trial: baseline characteristics of normocholesterolemic men with coronary artery disease and low levels of high-density lipoprotein cholesterol. Veterans Affairs Cooperative Studies Program High-Density Lipoprotein Intervention Trial Study Group. N Engl J Med 1999; 341:410-18.

27 Sniderman AD, Furberg CD, Keech A, Roeters van Lennep JE, Frohlich J, Jungner I, Walldius G. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin therapy. Lancet 2003; 361:777-80.

28 Choi HK, Hernán MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet 2002; 359:1173-7.

29 King AC, Haskell WL, Young DR, Oka RK, Stefanick ML. Long-term effects of varying intensities and formats of physical activity on participants rates, fitness, and lipoprotein in men and women aged 50 to 65 years. Circulation 1995; 91:2596-604.

30 Walsmith J, Abad L, Kehayias J, Roubenoff R. Tumor necrosis factor-α production is associated with less body cell mass in women with rheumatoid arthritis. J Rheumatol 2004; 31:23-9.

Chapter 5

IgM-rheumatoid factor, anti-cyclic citrullinated peptide and anti-citrullinated human fibrinogen antibodies decrease during treatment with the TNF blocker infliximab in patients with rheumatoid arthritis

M. Vis
W.H. Bos
G. Wolbink
A.E. Voskuyl
J. Twisk
R. van de Stadt
D. Hamann
B.A.C. Dijkmans
W.F. Lems

J Rheumatol 2008 Mar; 35(3):425-8

ABSTRACT

Objective: To investigate the effect of treatment with infliximab on serum levels of Rheumatoid factor (IgM-RF), antibodies against cyclic citrullinated peptide (anti-CCP) and antibodies against deiminated human fibrinogen (ACF) and their association with disease activity and disease duration in patients with rheumatoid arthritis (RA).

Methods: The study sample included 62 consecutive patients who were treated with infliximab for at least one year. IgM-RF, anti-CCP and ACF were measured at 0, 14, 30 and 46 weeks.

Results: Patients had a mean age of 54 years and median disease duration of 10 years and were predominantly female (95%). At baseline 63%, 77% and 82% of the patients were positive for IgM-RF, anti-CCP and ACF. In terms of percentages, the levels of IgM-RF were reduced by 64% at 46 weeks, while the anti-CCP and ACF levels were reduced by roughly 25%. The decrease in serum levels of these autoantibodies was not associated with the decrease in disease activity. The change in ACF was significantly related to disease duration, while the changes in IgM-RF or anti-CCP were not.

Conclusion: In conclusion, our study shows that in a cohort of RA patients who respond to infliximab therapy, all autoantibodies decreased significantly, but IgM-RF shows a larger decrease than anti-CCP or ACF. These changes in levels of autoantibodies are not directly related to the change in disease activity. Early in the disease ACF levels were best influenced by treatment with infliximab.

INTRODUCTION

Rheumatoid arthritis is a chronic polyarticular inflammatory disease which may lead to cartilage destruction and bone erosions. One of the characteristics of the disease is the presence of autoantibodies. Rheumatoid factor (IgM-RF) is an antibody, which targets the Fc fragment of IgG. IgM-RF is observed in about 75% of the RA patients, but it is also frequently observed in other inflammatory diseases [1, 2]. Antibodies against cyclic citrullinated peptide (anti-CCP) target multiple citrullinated proteins and are highly specific for RA [3-5]. Antibodies against a specific citrullinated peptide, deiminated fibrinogen (ACF), previously described as filaggrin antibodies, are as sensitive and specific for RA as the anti-CCP antibodies [6]. Deiminated fibrinogen has been detected in inflamed joints of RA patients, but also in non-RA inflamed joints [6, 7]. This could suggest that the antibody response against deiminated fibrinogen, but not the target, is specific for RA. These findings and their presence in early and even preclinical disease [8] suggests a role for anti-CCP and ACF antibodies in the pathogenesis of RA.

Furthermore, these antibodies (anti-CCP and ACF) appear to be a useful diagnostic tool and are predictive of disease progression and radiological damage [9]. Therefore, it is important to understand potential factors influencing these antibodies responses.

Several studies have described a decrease in IgM-RF serum level after disease modifying therapies [10-13]. Only few studies have examined the effect of aggressive RA treatment (anti-TNF) on the level of anti-CCP antibodies and these studies show conflicting results: 3 studies showed a decrease whereas 2 studies showed no change in anti-CCP titres during anti-TNF treatment [10-14].

Because of these conflicting results and to our knowledge the effect of treatment on serum levels of ACF antibodies has not been described, we investigated changes in IgM-RF, anti-CCP and ACF in a cohort of RA patients treated for one year with infliximab.

PATIENTS AND METHODS

Patients

Consecutive patients with RA, who were treated with infliximab for at least one year, were included. All patients fulfilled the ACR criteria for RA, had active disease (Disease Activity Score of 28 joints (DAS-28) ≥ 3.2) and had previously failed at least 2 DMARDs including methotrexate [15]. Previous infection with tuberculosis or cardiac failure NYHA class III and IV were contra-indications for treatment with infliximab. In total 80 patients were included into the cohort, but 18 patients dropped out within the first

year of treatment; 13 because of non-response, 3 because of side effects, and 2 patients died due to infliximab unrelated events. Thus, 62 patients were eligible for analyses. The baseline characteristics of the drop-out patients were comparable to the patients included into the study (data not shown).

Methods

Infusions with infliximab were given at 0, 2, 6 and 14 weeks and subsequently with 8 weeks interval. Infliximab was administered intravenously in a starting dose of 3 mg/kg. In patients with inadequate response as judged by the patient's rheumatologist the dosage of infliximab could be increased to 7.5 mg/kg.

Demographic data

The demographic data collected at baseline were recorded from medical history and patient's medical records. The following variables were collected: age, sex, disease duration, presence or absence of bony erosions, IgM-RF status (positive if IgM-RF > 30 u/L), current and previous use of disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids. The disease activity was measured at each visit using the DAS-28 score [16].

IgM-RF, CCP and ACF

Serum was collected on the morning before each infusion and stored immediately at -20 °C or lower until analyses. A total of 62 patients had serum available for evaluation. IgM-RF, anti-CCP and ACF were measured at 0, 14, 30 and 46 weeks.

The anti-CCP and IgM-RF was determined at the Jan van Breemen Institute, while the ACF was measured at Sanquin Research. IgM-RF was measured using an in-house ELISA. The anti-CCP level was measured using the anti-CCP-2 ELISA according to the manufacturer's recommendations (Axis-Shield). The ACF was measured as described previously [17]. In summary, microtitre plates where incubated for 1 hr with 10 ug/ml in vitro deiminated fibrinogen at RT. After five washes with PBS/0.2% Tween, plates were incubated with serum samples diluted 1:50 in PBS, 0.05% (vol/vol)Tween-20, 0.2% Gelatine (PTG) and were incubated for one hour. All assays were done in duplicate. After 5 more washes plates were incubated with a horseradish peroxidase (HRP) labelled mouse monoclonal antibody to IgG for 1hr. The plates were developed with tetra methyl benzidine (TMB) in 0.11 M NaAc and H_2O_2 , and the reaction was stopped with H_2SO_4. The plates were read at 540 nm wavelength, samples were considered positive when above the cut-off value determined previously (150 AU/ml).

Statistical analysis

In our analysis we only analyzed the positive patients for each test. Mann-Whitney test was used to compare changes in RF, anti-CCP and ACF between the different time-points.

As the distributions of RF, anti-CCP and ACF were not normal, a log transformation before statistical analyses was performed. Subsequently, Generalized Estimating Equations (GEE) analyses were performed. GEE is a regression analysis for longitudinal data modulating the course of the independent variables and dependent variables over time. GEE was used to investigate the course of these autoantibodies over time during treatment with infliximab and their longitudinal relationship with disease activity; DAS, CRP and ESR and with disease duration. To analyse the influence the effect of disease duration on change in autoantibodies we calculated an interaction-term: disease-duration * time-point. These analyses were performed using STATA version 7. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In total, 62 patients were included into the study: 59 (95%) women with a mean age of 54 years and median disease duration of 10 years (Table 1). The mean (SD) DAS-28 decreased from 5.4 (1.3) to 3.7 (1.6) at 14 weeks and 3.6 (1.5) at 30 and 46 weeks. Median ESR (mm/hr) decreased from 27 (2–85) at baseline to 16 (3–105 at 46 weeks) and the median CRP (mg/ml) from 11 (0–175) at baseline to 5 (0–178) at 46 weeks.

Table 1 Baseline characteristics of the 73 studied patients

Demography		
Age [years]	Mean (SD)	54 (12)
Gender [female]	n (%)	59 (81)
Disease duration [years]	Median (range)	10 (1–49)
Erosive disease	n (%)	57 (78)
Disease activity		
DAS-28	Mean (SD)	5.4 (1.3)
ESR [mm/hr]	Median (range)	27.0 (2.0–85.0)
CRP [mg/l]	Median (range)	11 (0.0–174.0)
Serum markers		
IgM-RF	Median (range)	53 (5–804)
Positive	n (%)	46 (63)
Anti-CCP	Median (range)	500 (0–16,640)
Positive	n (%)	56 (77)
ACF	Median (range)	616 (63–13,463)
Positive	n (%)	60 (82)

IgM-RF, anti-CCP and ACF

At baseline 63%, 77% and 82% of the patients were positive for IgM-RF, anti-CCP and ACF. All serum markers decreased significantly from baseline during one year treatment with infliximab as shown in Table 2.

Lipids and disease activity

The course of lipids over time was compared to the course of disease activity after adjustment for age and gender. GEE analyses yielded a significant inverse association between disease activity and lipid levels (Table 2). Changes in disease activity (DAS-28) were significantly related with changes in HDL- as well as total cholesterol levels. This means that if disease activity according to DAS-28 decreases one point, total cholesterol will raise 0.016 mmol/l (0.618 mg/dl) and HDL-cholesterol will raise 0.045 mmol/l (1.737 mg/dl), resulting in a more favorable i.e. lower, atherogenic index. Similarly, the individual inflammation parameter CRP was significantly inversely associated with both HDL- and total cholesterol levels. Moreover, the analyses showed that disease activity was inversely related with apo A-1, resulting in a lower, i.e. more favorable, apo B / apoA-1 ratio.

In terms of percentages the levels of IgM-RF were reduced by 64 percent at 46 weeks and the anti-CCP and ACF levels were reduced by roughly 25% (Figure 1). The percentage decrease in IgM-RF was statistically greater than the decrease in anti-CCP and ACF ($p<0.001$).

Nineteen patients of the 39 patients (49%), who at baseline were positive for IgM-RF, had a negative IgM-RF titre at 46 weeks. Only one of the 47 anti-CCP positive patients (2%) and three of the 54 ACF positive patients (6%) had a negative titre at 46 weeks.

The decrease in IgM-RF, anti-CCP and ACF levels during treatment with infliximab was compared to the course of the disease activity markers corrected for disease duration, gender and age: the analysis only showed a marginal association between ESR and anti-CCP levels, 1 mm/hr decrease in ESR is associated with a decrease of anti-CCP of 1.03 (SE 0.6) ($p<0.05$). We could not establish any other relationship between any of the disease activity markers and autoantibody levels.

Table 2 Changes in IgM-RF, anti-CCP and ACF during one year treatment with infliximab

	Baseline	14 weeks	30 weeks	46 weeks
IgM-RF	110 (34–804)	62 (13–627) *	51 (7–490) *	38 (8–465)*
Anti-CCP	1,232 (83–16,640)	1,220 (42–10,500)	1,050 (26–12,920)*	960 (30–11,880)*
ACF	754 (63–13,463)	687 (66–13,097) *	652 (69–10,289)*	539 (58–13,783)*

* $p<0.001$ vs. baseline calculated using GEE.

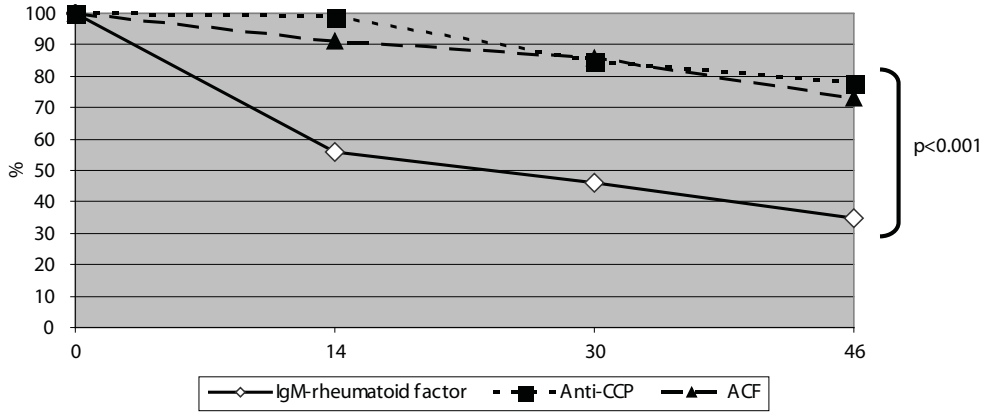

Figure 1 Percentage change from baseline in serum levels of IgM-RF, anti-CCP and ACF at 0, 14, 30 and 46 weeks during one year treatment with infliximab.

Likewise we also compared the course of autoantibodies corrected for disease duration. This analysis showed that the change in ACF was significantly related to disease duration but neither to IgM-RF nor anti-CCP. Interestingly, the ACF decreased more in patients with shorter disease duration ($p<0.05$).

DISCUSSION

In this study of RA patients who received infliximab for one year, IgM-RF, anti-CCP and ACF levels all decreased significantly. The decrease in IgM-RF was substantially larger than anti-CCP and ACF. Moreover, almost 50 percent of the IgM-RF positive patients at baseline turned negative during treatment with infliximab, while seroconversion of anti-CCP and ACF occurs in less than 10%.

The finding that anti-CCP decreases in our study differs from two other studies who reported unchanged anti-CCP levels during anti-TNF treatment. This difference could be due to the fact that we studied patients from a cohort of RA patients treated with infliximab for at least one year: these patients can all be classified as responders. Other studies observing responders showed a decrease in anti-CCP levels or showed a decrease in their responder subgroup [10, 12, 14]. On the other hand, studies investigating both responders and non-responders found no significant changes in anti-CCP [13].

Remarkably, we did not find a direct relationship between disease activity and changes in levels of autoantibodies, except a marginal relationship between ESR and

anti-CCP. This was surprising as the course of disease activity paralleled the change in autoantibodies. However, earlier studies investigating the effect of anti-rheumatic treatment on autoantibody titres, could not establish a consistent relationship between disease activity and changes in IgM-RF or anti-CCP [10, 14]. An explanation could be that autoantibody levels decrease in patients who respond to anti-rheumatic therapy, but there is no direct relationship with disease activity [10, 12, 15]. The effects of anti-TNF treatment in RA have been attributed to the induction of apoptosis of inflammatory cells [17]. Citrullination is a post-translational modification of proteins in the apoptotic process and therefore infliximab could influence the formation and presence of antibodies against citrullinated proteins by directly interfering with the regulation of the apoptotic process.

To our knowledge, this study was the first to investigate the response of ACF levels during treatment with a TNF-blocking agent. The ACF concentration showed a significant decrease during treatment with infliximab. ACF levels also decreased more in patients with shorter disease duration, possibly implying a window of opportunity for the treatment of RA. Antibodies to deiminated (citrullinated) fibrinogen have been implicated as a pathogenic factor for RA because of their high specificity, their presence in preclinical disease [18]. and their presence in rheumatoid synovium [7]. Lowering ACF levels through anti-rheumatic treatment may cause a disruption of the vicious circle of the proposed immunological conflict between citrullinated fibrin and its antibodies leading to self maintenance of RA synovitis [5].

In summary, our study shows that in a cohort of RA patients responding to infliximab treatment during one year of therapy, IgM-RF shows a larger decrease than anti-CCP or ACF, but all three autoantibodies decrease significantly. This decrease is not directly related to the change in disease activity but probably indirectly to a good clinical response. Early in the disease ACF levels were best influenced by treatment with infliximab.

REFERENCES

1 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; 31:1239-44.

2 Walker DJ, Pound JD, Griffiths ID, Powell RJ. Rheumatoid factor tests in the diagnosis and prediction of rheumatoid arthritis.Ann Rheum Dis 1986; 45:684-90.

3 Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, van Venrooij WJ. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000; 43:155-63.

4 Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998; 101:273-81

5 Chapuy-Regaud S, Sebbag M, Baeten D, Clavel C, Foulquier C, De Keyser F, Serre G. Fibrin deimination in synovial tissue is not specific for rheumatoid arthritis but commonly occurs during synovitides. J Immunol 2005; 174:5057-64

6 Masson-Bessiere C, Sebbag M, Girbal-Neuhauser E, Nogueira L, Vincent C, Senshu T, Serre G.J. The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. Immunol 2001; 166:4177-84.

7 Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. Arthritis Rheum 2004; 50:3485-94.

8 Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50:380-6.

9 Rantapää-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003; 48:2741-9.

10 Alessandri C, Bombardieri M, Papa N, Cinquini M, Magrini L, Tincani A, Valesini G. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFalpha therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. Ann Rheum Dis 2004; 63:1218-21.

11 Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 2004; 6:R264-R272.

12 Caramaschi P, Biasi D, Tonolli E, Pieropan S, Martinelli N, Carletto A, Volpe A, Bambara LM. Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment. Rheumatol Int 2005; 26:58-62.

13 De Rycke L, Verhelst X, Kruithof E, Van den Bosch F, Hoffman IE, Veys EM, De Keyser F. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. Ann Rheum Dis 2005; 64:299-302.

14 Braun-Moscovici Y, Markovits D, Zinder O, Schapira D, Rozin A, Ehrenburg M, Dain L, Hoffer E, Nahir AM, Balbir-Gurman A. Anti-cyclic citrullinated protein antibodies as a predictor of response to anti-tumor necrosis factor-alpha therapy in patients with rheumatoid arthritis. J Rheumatol 2006; 33:497-500.

15 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

16 van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996; 39:34-40.

17 Shen C, Maerten P, Geboes K, Van Assche G, Rutgeerts P, Ceuppens JL. Infliximab induces apoptosis of monocytes and T lymphocytes in a human-mouse chimeric model. Clin Immunol 2005; 115:250-9.

18 Nielen MM, van der Horst AR, van Schaardenburg D, van der Horst-Bruinsma IE, van de Stadt RJ, Aarden L, Dijkmans BA, Hamann D. Antibodies to citrullinated human fibrinogen (ACF) have diagnostic and prognostic value in early arthritis. Ann Rheum Dis 2005; 64:1199-204.

Chapter 6

Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis

G.J. Wolbink
M. Vis
W.F. Lems
A. Voskuyl
E. de Groot
M.T. Nurmohamed
H. de Vrieze
S. Stapel
P.P. Tak
L. Aarden
B. Dijkmans

Arthritis Rheum 2006 Mar; 54(3):711-5

ABSTRACT

Objective: Treatment of patients with infliximab, a chimeric monoclonal IgG1 antibody against tumor necrosis factor, may result in the formation of infliximab-specific IgG antibodies. This study evaluated the clinical significance of these antibodies in patients with rheumatoid arthritis (RA).

Methods: Anti-infliximab antibodies were measured using a newly developed radioimmunoassay in a cohort of 51 consecutive patients with RA treated with infliximab, with a follow-up of 1 year. In addition, serum infliximab levels were determined by enzyme-linked immunosorbent assay. The results were analyzed in relation to the clinical response to treatment according to the European League Against Rheumatism criteria.

Results: Antibodies against infliximab were detected in 22 patients (43%). Patients without detectable anti-infliximab antibodies (n=29 [57%]) were significantly more often classified as responders (20 of 29 [69%]) compared with patients with detectable anti-infliximab antibodies (8 of 22 [36%]; p=0.04). Three patients had an infusion-related allergic reaction, all of whom had detectable anti-infliximab antibodies.

Conclusion: In this study, nearly half of the RA patients treated with infliximab developed anti-infliximab antibodies within the first year of treatment. This seems to be clinically relevant, since development of anti-infliximab antibodies is associated with a reduced response to treatment.

INTRODUCTION

Treatment with infliximab provides great benefit to many patients with rheumatoid arthritis (RA) [1-3]. However, some patients have persistent active disease and others show loss of efficacy after prolonged treatment. Infliximab can induce the formation of antibodies to infliximab that may lead to side effects and loss of efficacy. Development of antibodies to infliximab is related to the dose of infliximab and is diminished by concomitant treatment with methotrexate (MTX) [1]. To what extent formation of antibodies to infliximab plays a role in clinical practice is unknown.

The Anti–Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) study group reported that 8% of patients receiving infliximab plus MTX developed antibodies to infliximab during the 102-week study period of the trial. However, despite the presence of antibodies, similar proportions of patients with antibodies to infliximab and without antibodies to infliximab achieved a 20% improvement response according to the American College of Rheumatology response criteria (ACR20) [4]. We have recently shown that a good response to treatment is associated with high serum trough levels of infliximab [5]. Conversely, serum trough levels of infliximab were below the limit of detection in patients prior to development of infusion-related allergic reactions. These findings suggest that formation of antibodies against infliximab might play a role in the efficacy of infliximab therapy in patients with RA.

To investigate the formation of antibodies against infliximab, we developed an antigen binding assay and measured levels of anti-infliximab antibodies in the serum of a cohort of 51 RA patients treated with infliximab, with a follow-up of 1 year. The presence of anti-infliximab IgG antibodies as well as levels of infliximab in the serum were related to clinical response and allergic reactions.

PATIENTS AND METHODS

Patients

Consecutive patients with RA who were receiving infliximab for RA at the Department of Rheumatology of Slotervaart Hospital in Amsterdam, The Netherlands, from April 2000 to January 2002 were included in this open, prospective observational study. Treatment of these patients was in accordance with the consensus statement on the initiation and continuation of tumor necrosis factor (TNF)-blocking therapy in RA [6]. All patients fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for RA [7], and all had evidence of

active disease, as indicated by a Disease Activity Score in 28 joints (DAS-28) of >3.2 despite earlier treatment with 2 disease-modifying antirheumatic drugs (DMARDs), including MTX, at a dosage of 25 mg weekly or at the maximum tolerable dosage (based on the Dutch guidelines for starting anti-TNF treatment).

All patients were given intravenous infusions of 3 mg/kg of infliximab at weeks 0, 2, and 6, and every 8 weeks thereafter. After 14 weeks of treatment, the treating rheumatologist was free to increase the dosage of infliximab to 7.5 mg/kg body weight in patients whose condition exhibited an inadequate response. Moreover, after 14 weeks, dosing intervals were kept stable at 8-week intervals. Concomitant medication, including MTX, was continued.

Disease activity was assessed using the DAS-28 [8] before each infusion. For assessment of clinical response, we used the European League Against Rheumatism (EULAR) response criteria [9]. Patients who stopped treatment or who needed a dosage escalation were regarded as non-responders. An infusion reaction was defined as any significant adverse event that occurred during the infusion or within 2 hours after the infusion.

Measurement of serum infliximab and anti-infliximab antibody levels

Serum samples were collected 1 hour prior to each infusion, for the assessment of serum infliximab and anti-infliximab antibodies. Infliximab levels in the serum were determined by enzyme-linked immunosorbent assay, as described elsewhere [5]. Anti-infliximab was detected with a newly developed radioimmunoassay. Infliximab-specific IgG was measured by an antigen binding test, essentially according to the procedure described by Aalberse et al. [10]. Briefly, serum (1 µl/test) was preincubated with agarose-immobilized protein A (1 mg/test; Pharmacia, Uppsala, Sweden) in Freeze buffer (CLB Sanquin, Amsterdam, The Netherlands). Non-bound serum components were removed by washing before ~1ng of 125I-labeled pepsin-treated infliximab was added. After overnight incubation, non-bound radiolabel was washed away and agarose-bound radioactivity was measured. High-radioactivity samples were retested at the appropriate dilutions. Test results were converted into arbitrary units (AU) per milliliter by comparison with dilutions of a reference serum. The cutoff level for a positive signal was set at 12 AU (mean +3 SD of the pretreatment values).

Statistical analysis

Differences between patient groups were analyzed by chi-square test or Mann-Whitney U test, as appropriate. The threshold for significance was set at a p-value of less than 0.05. To analyze the change in the DAS-28 among patients with and without anti-infliximab antibodies after 1 year of treatment, we used the last observation carried

forward for patients who stopped treatment or who had received an increased dosage of infliximab.

RESULTS

Characteristics of the cohort

The majority of the 51 patients who entered the study were women (82%). The mean ± SD age of the patients was 56 ± 13 years and the mean ± SD disease duration was 12 ± 9 years. Seventy percent of the patients were rheumatoid factor positive. The mean number of DMARDs received before infliximab treatment was 3.7. Forty-four patients (86%) were receiving concomitant MTX, with a mean dosage of 15 mg/every week, while 3 patients were receiving azathioprine and 1 patient was receiving concomitant cyclosporine. The remaining 3 patients were not taking concomitant immunosuppressive drugs. At study entry, all patients had active disease, as indicated by a mean ± SD DAS-28 of 6.0 ± 1.3.

Clinical response

After 1 year of follow-up, 28 patients (55%) were classified as treatment responders according to the EULAR response criteria, without having needed an increase in the infliximab dosage. After 14 weeks of treatment, the mean ± SD DAS-28 had improved to 4.7 ± 1.7. Eight patients stopped treatment before the end of 1 year of therapy. Two patients stopped receiving infliximab after developing an infusion-related allergic reaction. Two patients stopped treatment because of skin problems, 1 patient because of edema, and 3 patients because of treatment inefficacy. Six patients received a dosage escalation.

Detection of anti-infliximab antibodies

Prior to treatment, the anti-infliximab concentration was below 10 AU/ml in all 51 samples. At follow-up, serum samples from 22 patients were found to be positive for anti-infliximab antibodies. One patient was antibody positive at week 6 after start of treatment, 6 patients at week 14, 6 patients at week 22, 2 patients at week 30, 4 patients at week 38, and 3 patients at week 46. In most of these patients, the titer of anti-infliximab antibodies increased during continuation of treatment. In 5 patients with relatively low titers of anti-infliximab (25 AU/ml, 93 AU/ml, 18 AU/ml, 25 AU/ml, and 64 AU/ml), the anti-infliximab levels became undetectable during follow-up, while in 3 patients, anti-infliximab antibodies fell below the level of detection after

dosage escalation. In these latter 3 patients, we observed an increase in the serum trough levels of infliximab just above the limit of detection (0.1 mg/liter) that coincided with an improvement in the DAS-28.

Patients without detectable anti-infliximab antibodies were significantly more often classified as responders when compared with patients with detectable anti-infliximab antibodies (20 of 29 without antibodies [69%] versus 8 of 22 with antibodies [36%] considered responders; p=0.04) (Figure 1). Patients without detectable anti-infliximab antibodies had significantly more improvement in the DAS-28 than did patients with detectable anti-infliximab antibodies (mean ± SD decrease in the DAS-28 1.9 ± 1.2 versus 0.9 ± 1.8; p=0.02). The maximum anti-infliximab titer was significantly higher in patients classified as non-responders (median 42 AU/ml, interquartile range 8–310 AU/ml) compared with patients classified as responders (median 9 AU/ml, interquartile range 6–17 AU/ml) (p=0.025) (Figure 2).

In 3 patients, there was an infusion-related allergic reaction. In all 3 of these patients, the infusion reactions consisted of tachycardia, erythema, and shortness of breath. These patients were treated with antihistamines, and full recovery occurred within a few hours. Two of the patients stopped further treatment, whereas 1 patient continued treatment without further infusion reactions. Anti-infliximab antibodies

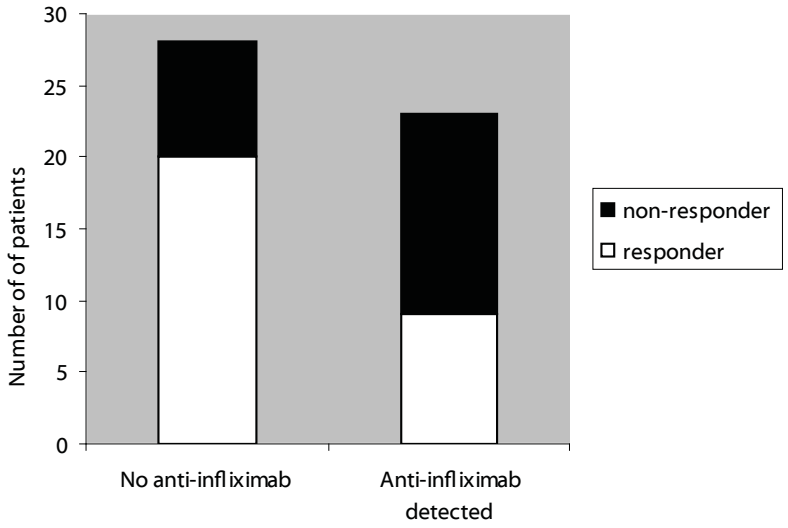

Figure 1 Presence or absence of detectable anti-infliximab antibodies in relation to response to treatment (according to the European League Against Rheumatism criteria) with infliximab. Patients without detectable anti-infliximab were significantly more often classified as responders compared with patients with detectable anti-infliximab (20 of 29 [69%] versus 8 of 22 [36%]; p=0.04).

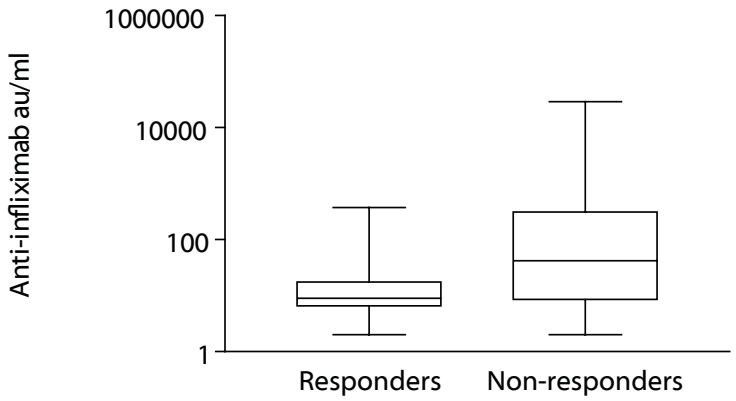

Figure 2 Maximum anti-infliximab titers in patients classified as responders versus patients classified as non-responders to infliximab therapy. The maximum anti-infliximab titer was significantly higher in non-responders (median 42 arbitrary units [AU]/ml, interquartile range 8–310) compared with responders (median 9 AU/ml, interquartile range 6–17) (p=0.025). Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles.

were detected in the serum of all 3 of these patients (786 AU/ml, 748 AU/ml, and 64 AU/ml, respectively). However, most of the patients with anti-infliximab antibodies, including 2 patients with the highest titers of anti-infliximab (1,359 AU/ml and 29,133 AU/ml), did not develop an allergic infusion reaction.

The development of anti-infliximab antibodies coincided with a decrease in serum trough levels of infliximab prior to the detection of anti-infliximab. Detection of antibodies against infliximab occurred only in samples with undetectable serum trough levels of infliximab. An example is shown in Figure 3. Mean serum trough levels of infliximab at 8 weeks after administration of 3 mg/kg infliximab were significantly lower in patients with anti-infliximab antibodies compared with patients without such antibodies (0.2 mg/liter versus 1.5 mg/liter; $p<0.001$).

Baseline characteristics of the patients with and without anti-infliximab antibodies were similar. The mean MTX dose in patients with and without anti-infliximab antibodies was similar. None of the 3 patients receiving azathioprine as concomitant therapy had detectable anti-infliximab antibodies. Antibodies to infliximab were detected in 2 of the 3 patients who did not receive concomitant medication. All of these patients who had not taken concomitant medication were considered non-responders to the infliximab regimen.

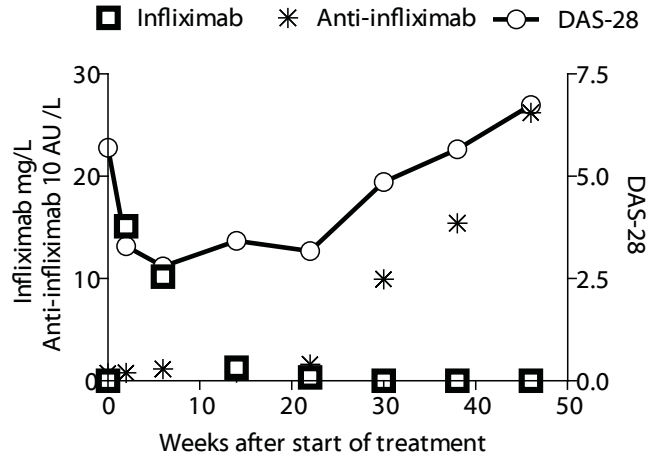

Figure 3 Trough levels of infliximab in the serum (□), levels of anti-infliximab (✳), and the Disease Activity Score in 28 joints (DAS-28) (○) in a rheumatoid arthritis patient treated with infliximab. After an initial improvement of disease activity, the patient had a relapse of disease activity that coincided with a decrease in the serum trough levels of infliximab and an increase in the anti-infliximab titer. AU, arbitrary units.

DISCUSSION

The results presented herein show that almost half of the 51 patients with RA who were treated with 3 mg/kg infliximab every 8 weeks developed detectable anti-infliximab IgG antibodies within the first year of treatment. Moreover, the presence of these antibodies was associated with a reduced response to treatment.

In the ATTRACT study, 8% of patients receiving infliximab plus MTX developed antibodies to infliximab during the 102-week study period. It was reported that despite the presence of antibodies, similar proportions of patients with antibodies to infliximab and without antibodies to infliximab achieved an ACR20 response [2]. In patients with Crohn's disease, 61% of patients treated with infliximab developed anti-infliximab antibodies [11]. It has been demonstrated that RA patients who respond to therapy have higher serum trough levels of infliximab compared with patients who do not respond to therapy [5]. Indeed, serum trough levels of infliximab were undetectable after an 8-week treatment interval in patients who developed an infusion-related allergic reaction.

The difference between the findings of this study and those reported by the ATTRACT study group might be explained by the fact that in our study, all patients started treatment on the 3 mg/kg dosage every 8 weeks, while 75% of the patients in the ATTRACT study received higher dosages. In addition, some of the difference might be attributable to differences in the assays used; thus, a formal comparison between the assays is warranted.

Experiments with animal models have shown that induction of an immune response to therapeutic antibodies is associated with an accelerated clearance of the antibody [12]. Patients with detectable anti-infliximab have lower mean serum trough levels of infliximab compared with patients without anti-infliximab antibodies. This indicates that anti-infliximab antibody formation accelerates the clearance of infliximab from the circulation.

Interestingly, we observed that in some patients with anti-infliximab antibodies and an inadequate response to treatment, continuation of treatment with higher dosages of infliximab resulted in decreased levels of anti-infliximab antibodies. This might be attributable to induction of immune tolerance, similar to what is seen in patients with hemophilia who develop antibodies to factor VIII. Alternatively, it could be the result of overdosing the capacity of the immune system to produce anti-infliximab antibodies. It can be speculated that continuation of treatment with increased dosages of infliximab is effective in patients with low anti-infliximab antibody titers, whereas those patients with high titers of anti-infliximab antibodies probably benefit more from switching to other TNF-blocking agents.

Infliximab may induce infusion-related allergic reactions. In all 3 patients who developed an infusion-related reaction, high levels of anti-infliximab antibodies were detected. It is remarkable that most of the patients with anti-infliximab antibodies did not have a clinically overt allergic response. This is not simply related to the titer of the antibodies, since 2 patients who had the highest antibody titers did not have clinically overt infusion reactions. The fact that 2 of the 3 patients who received infliximab without concomitant immunosuppressive medication developed anti-infliximab antibodies illustrates the importance of adequate concomitant immunosuppressive therapy to prevent formation of antibodies to infliximab.

Many patients with RA exhibit persistent disease activity despite having received infusions of infliximab. The response to infliximab therapy is related to the level of infliximab in the serum and the presence of anti-infliximab antibodies, and shows a large interindividual variation. Further investigation into the mechanisms that determine serum concentrations of infliximab and formation of anti-infliximab antibodies may help to optimize this treatment.

REFERENCES

1 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti–tumor necrosis factor monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998; 41:1552-63.

2 Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, St Clair EW, Keenan GF, van der Heijde D, Marsters PA, Lipsky PE; Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum 2004; 50:1051-65.

3 St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, Keystone EC. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 2002; 46:1451-9.

4 Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot R Jr, Paulus H, Strand V, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995; 38:727-35.

5 Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BAC, Aarden L. Relation between serum trough infliximab levels, pre-treatment CRP levels and clinical response to infliximab treatment in rheumatoid arthritis. Ann Rheum Dis 2005; 64:704-7.

6 Smolen J, Breedveld F, Burmester G, Combe B, Emery P, Kalden J, Klareskog L, Maini RV, Numo R, van de Putte LBA, van Riel PLCM, Rodriguez-Valverde V. Consensus statement on the initiation and continuation of TNF blocking therapies in rheumatoid arthritis. Ann Rheum Dis 2000; 59:504-5.

7 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-24.

8 Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight–joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38:44-8.

9 van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis: comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism criteria. Arthritis Rheum 1996; 39:34-40.

10 Aalberse RC, Dieges PH, Knul-Bretlova V, Vooren P, Aalbers M, van Leeuwen J. IgG4 as a blocking antibody. Clin Rev Allergy 1983; 1:289-302.

11 Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, Carbonez A, Rutgeerts P. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med 2003; 348:601-8.

12 LoBuglio AF, Wheeler RH, Trang J, Haynes A, Rogers K, Harvey EB, Sun L, Ghrayeb J, Khazaeli MB. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. Proc Natl Acad Sci U S A 1989; 86:4220-4.

Chapter 7

Adverse events in patients with rheumatoid arthritis treated with infliximab in daily clinical practice

N. Neven
M. Vis
A.E. Voskuyl
G.J. Wolbink
M.T. Nurmohamed
B.A.C. Dijkmans
W.F. Lems

Ann Rheum Dis 2005 Apr; 64(4):645-6

INTRODUCTION

Infliximab is highly effective and relatively safe for the treatment of patients with rheumatoid arthritis (RA) in clinical trials [1-5]. This prospective cohort study was undertaken to determine adverse events, in particular, infections in patients with RA treated with infliximab in daily clinical practice.

METHODS AND RESULTS

We treated 168 patients with RA between 1 April 2000 and 1 October 2002, 82% female, with a median disease duration of 10 years (range 1–49). Inclusion criteria were 28 joint count Disease Activity Score (DAS-28) of >3.5 and failure of two disease modifying antirheumatic drugs, including methotrexate. Patients with heart failure or with a malignancy 5 years before screening were excluded. After the alert about tuberculosis, 6 patients starting with infliximab treatment were screened for that disease.

All patients were treated with an initial infliximab dose of 3 mg/kg (weeks 0, 2, 6, and subsequently, every 8 weeks). When the response was insufficient — that is, a decrease in DAS-28 <1.2 compared with baseline on two subsequent occasions, the dose could be increased to 7.5 mg/kg. The median duration of treatment was 0.86 years (range 0–2.5); the median number of infusions used was 7 (range 1–18). Methotrexate and prednisone were used by 92% and 50% of the patients, respectively.

Patients were systematically asked about events and, explicitly, about infections at each visit. All events occurring during the infliximab treatment period were interpreted as adverse events.

The most common mild adverse event was short lived headache. Early allergic reactions were seen in 12 patients (0.08/patient year), but none developed severe cardiopulmonary problems. Some cases of heart failure (n=2), neuropathy (n=1), and malignancy (n=2) were observed. Two patients died during the study, one of a cerebrovascular accident and one of unknown cause.

Patients frequently (43–57%, depending on the definition used) had infections, most commonly from the upper respiratory tract and the lower urinary tract (Table 1). One case of tuberculosis was seen. The number of clinically important infections was 0.59/patient year, whereas serious infections were found in 0.08/patient year.

Compared with patients receiving low dose infliximab, significantly ($p<0.05$) more patients with the increased dose had clinically important infections (including serious infections), but other adverse events, demographic characteristics, and drug use between the groups were comparable. After correction for treatment duration

Table 1 Occurrence of adverse events in 168 patients with RA treated with infliximab

				3 mg/kg infliximab (n=132)			3→7.5 mg/kg infliximab (n=36)		
Adverse events	No (%)	Events	Events/ patient year	No (%)	Events	Events/ patient year	No (%)	Events	Events/ patient year
Any adverse event	122 (73)	208	1.44	89 (67)	152	1.40	30 (83)	56	1.30
All infections*	96 (57)	153	1.06	69 (52)	109	1.01	27 (75)	44	1.02
Clinical infections†	72 (43)	93	0.64	50 (38)¶	65	0.41 ¶¶	22 (61)¶	28	0.65¶
Clinically important infections‡	60 (36)	85	0.59	42 (32)¶	59	0.55	18 (50)¶	26	0.60
Antibiotics (po)	50 (30)	74	0.51	35 (27)¶	51	0.47	15 (42)¶	23	0.53
Serious infections§	10 (6)	11	0.08	7 (5)	8	0.07	3 (8)	3	0.07

* All infections defined as: objective and non-objective infections as reported by patient, clinical physician, or general physician, including unconfirmed upper respiratory tract infection and unconfirmed lower urinary tract infection; † Clinical infections as considered present by a physician — that is, sinusitis, upper respiratory tract infection, pneumonia, pyelonephritis, bacteriaemia/septicaemia, lower urinary tract infection, gastroenteritis, skin and soft tissue infections with relevant findings of physician; ‡ Clinically important infections as defined by objective infection, eventually requiring oral antibiotic treatment; § Serious infection as defined by admission to hospital and/or requiring IV antibiotic treatment (in majority pneumoniae); ¶ Difference significant, p-value <0.05.

with infliximab, the rate of clinical infections was significantly higher in the group receiving the increased dose. However, after correction for treatment duration, clinically important infections were not significantly more common in the group receiving the increased dose.

DISCUSSION

Our study has shown that infection is the most common adverse event of infliximab treatment in daily practice. Clinical infections and clinically important infections were found more frequently in patients receiving high dose infliximab, without proven causality.

The occurrence of infections in our study is in the same range as that described in (randomised) clinical trials of infliximab [1, 3, 5, 7]. However, the incidence of infection in our study was much higher than those described in a population based study of patients with RA not treated with infliximab, 64 versus 32 events per patient per year [8].

There is evidence that a higher risk for infections occurs with a higher RA activity [9]. It is reasonable to suppose that patients with a dose increase had greater disease activity than those treated with only low dose infliximab. We are unable to comment on whether the higher incidence of infections is associated with a high disease activity or with the strong immunosuppressive action of infliximab, or both.

In conclusion, infliximab can be used safely in daily clinical practice, but both doctors and patients should be aware of the (infection) risks, especially in patients receiving a higher dose (>3 mg/kg) of infliximab, in order to anticipate and minimise these risks.

REFERENCES

1 Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999; 354:1932-9.

2 Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN; Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000; 343:1594-602.

3 Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. Lancet 1994; 344:1105-10.

4 Harriman G, Harper LK, Schaible TF. Summary of clinical trials in rheumatoid arthritis using infliximab, an anti-TNFalpha treatment. Ann Rheum Dis 1999; 58(suppl I):I61-I64.

5 Kroesen S, Widmer AF, Tyndall A, Hasler P. Serious bacterial infections in patients with rheumatoid arthritis under anti-TNF-alpha therapy. Rheumatology (Oxford) 2003; 42:617-21.

6 Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001; 345:1098-104.

7 Fitzcharles MA, Clayton D, Menard HA. The use of infliximab in academic rheumatology practice: an audit of early clinical experience. J Rheumatol 2002; 29:2525-30.

8 Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum 2002; 46:2287-93.

9 Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Predictors of infection in rheumatoid arthritis. Arthritis Rheum 2002; 46:2294-300.

Chapter 8

The effect of intravenous pamidronate versus oral alendronate on bone mineral density in patients with osteoporosis

M. Vis
I.E.M. Bultink
B.A.C. Dijkmans
W.F. Lems

Osteoporos Int 2005 Nov; 16(11):1432-5

ABSTRACT

Intravenous pamidronate is frequently used for the treatment of osteoporosis in patients who cannot tolerate oral bisphosphonates. The aim of the present study was to compare the changes in bone mineral density (BMD) after 1 year of treatment with either oral alendronate or intravenous pamidronate in patients with osteoporosis. We studied 40 consecutive patients starting treatment for osteoporosis: 20 received oral alendronate 10 mg/day and 20 received intravenous pamidronate 60 mg/3 months. Patients were started on intravenous pamidronate in the case of intolerance (within 1 month of start of treatment) of an oral bisphosphonate or in the case of contraindications for an oral bisphosphonate. BMD (spine and total hip) was measured with dual X-ray absorptiometry (DEXA) at the start of treatment and after 1 year. The BMD of the lumbar spine increased by 4.0% ($p<0.05$ vs baseline) in both groups, and the BMD of the hip increased by 3.3% and 2.9% ($p<0.05$ vs baseline) in the alendronate and pamidronate groups, respectively. The increases in BMD of the vertebral spine and the total hip after 1 year are comparable in the alendronate and pamidronate groups. We conclude that intravenous pamidronate can be used successfully as an alternative treatment in patients with gastrointestinal intolerance of an oral bisphosphonate.

INTRODUCTION

Osteoporosis is a systemic bone disease characterized by loss of bone mineral density (BMD) and deterioration of micro-architecture. This results in fragile bones with an increased risk of fractures [1].

Bisphosphonates are the most frequently used drugs for the treatment and prevention of osteoporosis. These drugs are synthetic analogues of pyrophosphate and inhibit osteoclast-mediated bone resorption [2, 3]. All bisphosphonates have demonstrated an increase in BMD in postmenopausal osteoporosis [4-7]. However, only alendronate and risedronate have a proven effect in the reduction of vertebral and peripheral fractures [5, 7].

Gastrointestinal complaints are the most common side effects of oral bisphosphonates [5, 7-10]. The large randomized clinical trials (RCTs) investigating oral daily alendronate in postmenopausal women with osteoporosis showed incidence rates of upper gastrointestinal adverse events of between 22% and 48% [8-10]. Although the incidence rates in the placebo groups were comparable, these data still indicate that dyspepsia is an important issue in the treatment of patients with osteoporosis. In addition to oral administration, pamidronate can also be given through intravenous infusion. Therefore, pamidronate is frequently used in daily practice for patients who cannot tolerate oral bisphosphonates, in spite of the lack of anti-fracture data during intravenous use of pamidronate.

Prospective studies in the treatment of naïve osteoporosis patients, comparing an intravenous bisphosphonate with an oral one, are lacking. Since it is inconceivable that such a randomized study will be performed, we observed the changes in BMD (spine and hip) in daily clinical practice during 1 year of treatment with either alendronate 10 mg/day or intravenous pamidronate 60 mg/3 months.

PATIENTS AND METHODS

Since 2000, Slotervaart Hospital (Amsterdam, the Netherlands) has had an outpatient clinic for patients with osteoporosis. Data are gathered prospectively and systematically in this clinical practice setting. Patients referred to this clinic are screened routinely for osteoporosis and receive treatment when indicated. Patients who are treated with anti-osteoporotic drugs return for a routine follow-up visit each year.

Patients

Forty consecutive patients attending the osteoporosis clinic who were started on treatment with either oral alendronate 10 mg/day or intravenous pamidronate 60 mg/3 months were included in the study. Twenty patients were allocated to each group. The first choice for bisphosphonate treatment of osteoporosis was oral alendronate. Intravenous pamidronate was given to patients with gastrointestinal contraindications for oral bisphosphonate (n=9) or with intolerance of oral bisphosphonate (within 1 month of initiation of bisphosphonate therapy, n=11). Patients with previous treatment for longer than 1 month with anti-osteoporosis drugs were not included.

If the dietary intake of calcium was below 1,000 mg/day, patients received supplementation with calcium. Thirteen patients in the alendronate group and 17 in the pamidronate group received calcium supplementation. Vitamin D supplementation (colecalciferol 400 U/day) was given in patients whose serum 25(OH) vitamin D level was below 30 nmol/l at baseline. In each group seven patients received supplementation with colecalciferol.

Assessments

Demographic data and data on risk factors for osteoporosis were collected by questionnaire at baseline and after 1 year. Data collected comprised: age, body mass index (BMI), menopausal status, history of fractures, family (first degree) history of fractures (vertebral and peripheral fractures), thyroid function, kidney function, current medication (including prednisone) and history of prednisone use.

BMD was measured with dual X-ray absorptiometry (DEXA) at the lumbar-spine (L1–L4) and the total hip at the start of treatment and after 1 year. All BMD measurements were performed with the same equipment (Hologic 4500, Waltham, MA, USA).

Statistics

To compare the distribution of risk factors for osteoporosis between groups we used Pearson's chi-square test for dichotomous variables and Student's t-test for continuous variables. Changes in BMD between the groups and in the groups were compared by an independent and paired Student's t-test, respectively. The data were analyzed with the SPPS 11.1 software package. A p-value below 0.05 was considered statistically significant.

RESULTS

The patient characteristics are shown in Table 1. There were no significant differences between the groups in age, BMI, gender, use of prednisone, thyroid and kidney function (data not shown), history of fractures or family history of fractures. The BMD of the spine and the total hip at baseline were also comparable between the alendronate and pamidronate groups (Table 2).

There was no difference in risk factors and baseline BMD between the patients treated with intravenous pamidronate because of gastrointestinal co-morbidity and those with gastrointestinal intolerance of oral bisphosphonate.

Table 1 Epidemiological data and risk factors for osteoporosis in the alendronate and pamidronate groups

Parameter		Alendronate 10 mg/day per os n=20	Pamidronate 60 mg/3 months intravenous n=20	
Age	Mean (SD)	68.5 (15.4)	67.3 (15.7)	NS
BMI	Mean (SD)	25.9 (5.5)	23.7 (3.2)	NS
Gender (female)	n (%)	15 (75)	16 (67)	NS
Post menopausal	n (%)	14 (93)	15 (88)	NS
Fractures (history) (vertebral and peripheral)	n (%)	11 (55)	8 (40)	NS
Familial fractures	n (%)	8 (40)	10 (50)	NS
Calcium intake (mg/day)	Mean (SD)	1,055 (465)	1,070 (470)	NS
Vitamin D concentration (nmol/l)	Median (range)	60 (3–120)	57 (5–110)	NS
Prednisone (history)				
Ever used prednisone	n (%)	5 (25)	5 (25)	
Current use of prednisone	n (%)	5 (25)	6 (30)	NS
Never used prednisone	n (%)	10 (50)	9 (45)	

Table 2 Baseline BMD data (mean [SD]) in the alendronate and pamidronate groups

Parameter	Alendronate group	Pamidronate group	
Vertebral spine			
BMD (g/cm^2)	0.819 (0.167)	0.748 (0.150)	NS
t-score	-2.05 (1.18)	-2.79 (1.36)	NS
z-score	-0.53 (1.33)	-1.06 (1.47)	NS
Total hip			
BMD (g/cm^2)	0.720 (0.133)	0.704 (0.135)	NS
t-score	-1.55 (0.73)	-2.05 (1.10)	NS
z-score	-0.59 (0.88)	-0.81 (1.13)	NS

BMD change

The increase in BMD of the vertebral spine in both groups was comparable. The BMD of the lumbar spine increased significantly in both groups: 0.032 g/cm^2 (+4.0%, p<0.001 vs baseline) in the alendronate group and 0.028 g/cm^2 (+4.0%, p<0.05 vs baseline) in the pamidronate group. According to total hip measurements the BMD increased significantly in both the alendronate and the pamidronate groups: 0.025 g/cm^2 (3.3%) and 0.018 g/cm^2 (+2.9%) (both p<0.05 vs baseline), respectively. The difference in change in BMD of the hip between the alendronate and pamidronate groups was not statistically significant.

DISCUSSION

This study shows that, in patients visiting our osteoporosis clinic, treatment with intravenous pamidronate is as effective as treatment with oral alendronate, measured by the effect on BMD. Therefore, we suggest that intravenous pamidronate is an alternative for patients who cannot tolerate oral bisphosphonate or have gastrointestinal contraindications for these drugs. We realize that our study population may be subject to selection bias; patients receiving intravenous pamidronate were selected because of gastrointestinal problems. It is possible that those patients differed from the alendronate-treated patients who did not have gastrointestinal intolerance or co-morbidity. However, the positive effects on BMD in the alendronate group were also observed in the pamidronate group, which suggests that pamidronate is an acceptable alternative to alendronate in these patients.

Upper gastrointestinal adverse events are common during treatment with oral bisphosphonates [4]. After the introduction of alendronate a post-marketing surveillance study showed that alendronate could cause chemical esophagitis, with severe ulceration [8, 11]. Based on the data presented in that report, the dosing instructions were altered to reduce esophagitis. Those instructions greatly reduced the number of upper gastrointestinal adverse events. Large RCTs on the use of oral bisphosphonates suggest that the incidence rates of upper gastrointestinal complaints in alendronate and placebo are now roughly the same [8-10]. However, these data apply to large cohorts of selected patients. In daily clinical practice the intolerance of oral bisphosphonates is still a major issue for the physician treating osteoporosis patients. Therefore, we frequently use intravenous pamidronate in those osteoporosis patients.

Low compliance is an issue in the treatment of all chronic diseases [12]. Osteoporosis is a chronic disease and anti-osteoporotic drugs have to be taken for a long period of time. Poor adherence limits the effect of bisphosphonates, which makes compliance

an important problem in treatment with bisphosphonates [13]. A few studies have investigated the compliance with bisphosphonates for the treatment of osteoporosis in daily practice. One study found a discontinuation rate as high as 30% during the first half year of treatment with oral bisphosphonates [14]. Intravenous administration of pamidronate provides certainty that patients receive their anti-osteoporotic treatment and gives more control over patients' compliance.

We investigated the effect of bisphosphonates on BMD. Although BMD is used as a surrogate endpoint for studies in osteoporosis, the primary goal of treatment remains fracture reduction. However, if the efficacy of anti-osteoporotic agents on fracture rates is to be to properly evaluated, a very large study population is needed. To show a clinically and statistically significant difference between two anti-osteoporotic drugs, a study would have to include approximately 100,000 patients. A study with such a sizable population is almost impossible to perform [15]. In addition, a low BMD is the most important risk factor to predict fractures. Although weak, associations between changes in BMD and reductions in vertebral and peripheral fractures have been shown during treatment with bisphosphonates [16, 17]. Our study was performed in small groups and only comprises BMD data; nevertheless, this is the first prospective study comparing intravenous bisphosphonate with an oral bisphosphonate in one study.

In summary, the effect on BMD of oral alendronate and intravenous pamidronate is comparable. Therefore, we conclude that intravenous pamidronate is an acceptable alternative to oral bisphosphonates in the treatment of osteoporosis for patients who cannot tolerate an oral bisphosphonate or have gastrointestinal contraindications for these drugs.

REFERENCES

1 Riggs BL, Melton LJ III. The prevention and treatment of osteoporosis. N Engl J Med 1992; 327:620-7.

2 Widler L, Jaeggi KA, Glatt M, Muller K, Bachmann R, Bisping M, Born AR, Cortesi R, Guiglia G, Jeker H, Klein R, Ramseier U, Schmid J, Schreiber G, Seltenmeyer Y, Green JR. Highly potent geminal bisphosphonates. From pamidronate disodium (Aredia) to zoledronic acid (Zometa). J Med Chem 2002; 45:3721-38.

3 Fitton A, McTavish D. Pamidronate. A review of its pharmacological properties and therapeutic efficacy in resorptive bone disease. Drugs 1991; 41:289-318.

4 Thiebaud D, Burckhardt P, Melchior J, Eckert P, Jacquet AF, Schnyder P, Gobelet C. Two years effectiveness of intravenous pamidronate (APD) versus oral fluoride for osteoporosis occurring in the postmenopause. Osteoporos Int 1994; 4:76-83.

5 Reginster J, Minne HW, Sorensen OH, Hooper M, Roux C, Brandi ML, Lund B, Ethgen D, Pack S, Roumagnac I, Eastell R. Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis. Vertebral Efficacy with Risedronate Therapy (VERT) Study Group. Osteoporos Int 2000; 11:83-91.

6 Peretz A, Body JJ, Dumon JC, Rozenberg S, Hotimski A, Praet JP, Moris M, Ham H, Bergmann P. Cyclical pamidronate infusions in postmenopausal osteoporosis. Maturitas 1996; 25:69-75.

7 Liberman UA, Weiss SR, Broll J, Minne HW, Quan H, Bell NH, Rodriguez-Portales J, Downs RW Jr, Dequeker J, Favus M. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. N Engl J Med 1995; 333:1437-43.

8 Pols HA, Felsenberg D, Hanley DA, Stepan J, Munoz-Torres M, Wilkin TJ, Qin-sheng G, Galich AM, Vandormael K, Yates AJ, Stych B. Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT study. Foxamax International Trial Study Group. Osteoporos Int 1999; 9:461-8.

9 Hosking D, Chilvers CE, Christiansen C, Ravn P, Wasnich R, Ross P, McClung M, Balske A, Thompson D, Daley M, Yates AJ. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. Early Postmenopausal Intervention Cohort Study Group. N Engl J Med 1998; 338:485-92.

10 Bauer DC, Black D, Ensrud K, Thompson D, Hochberg M, Nevitt M, Musliner T, Freedholm D. Upper gastrointestinal tract safety profile of alendronate: the fracture intervention trial. Arch Intern Med 2000; 160:517-25.

11 de Groen PC, Lubbe DF, Hirsch LJ, Daifotis A, Stephenson W, Freedholm D, Pryor-Tillotson S, Seleznick MJ, Pinkas H, Wang KK. Esophagitis associated with the use of alendronate. N Engl J Med 1996; 335:1016-21.

12 Miller NH. Compliance with treatment regimens in chronic asymptomatic diseases. Am J Med 1997; 102:43-9.

13 Yood RA, Emani S, Reed JI, Lewis BE, Charpentier M, Lydick E. Compliance with pharmacologic therapy for osteoporosis. Osteoporos Int 2003; 14:965-8.

14 Ettinger B, Pressman A, Schein J. Clinic visits and hospital admissions for care of acid-related upper gastrointestinal disorders in women using alendronate for osteoporosis. Am J Manag Care 1998; 4:1377-82.

15 Kanis JA, Oden A, Johnell O, Caulin F, Bone H, Alexandre JM, Abadie E, Lekkerkerker F. Uncertain future of trials in osteoporosis. Osteoporos Int 2002; 13:443-9.

16 Khosla S. Surrogates for fracture endpoints in clinical trials. J Bone Miner Res 2003; 18:1146-9.

17 Hochberg MC, Greenspan S, Wasnich RD, Miller P, Thompson DE, Ross PD. Changes in bone density and turnover explain the reductions in incidence of nonvertebral fractures that occur during treatment with antiresorptive agents. J Clin Endocrinol Metab 2002; 87:1586-92.

Chapter 9

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 TNFα-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 NFκb 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 OSTRA-group. Five years ago the OSTRA-group 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 bisphophonates 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 bisphophonates, 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 OSTRA-cohort, 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 essays 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.

REFERENCES

1 Incidence of vertebral fracture in Europe: results from the European Prospective Osteoporosis Study (EPOS). J Bone Miner Res 2002; 17:716-24.

2 Nevitt MC, Cummings SR, Stone KL, Palermo L, Black DM, Bauer DC, Genant HK, Hochberg MC, Ensrud KE, Hillier TA, Cauley JA. Risk factors for a first-incident radiographic vertebral fracture in women > or = 65 years of age: the study of osteoporotic fractures. J Bone Miner Res 2005; 20:131-40.

3 Ismail AA, Pye SR, Cockerill WC, Lunt M, Silman AJ, Reeve J, Banzer D, Benevolenskaya LI, Bhalla A, Bruges Armas J, Cannata JB, Cooper C, Delmas PD, Dequeker J, Dilsen G, Falch JA, Felsch B, Felsenberg D, Finn JD, Gennari C, Hoszowski K, Jajic I, Janott J, Johnell O, Kanis JA, Kragl G, Lopez Vaz A, Lorenc R, Lyritis G, Marchand F, Masaryk P, Matthis C, Miazgowski T, Naves-Diaz M, Pols HA, Poor G, Rapado A, Raspe HH, Reid DM, Reisinger W, Scheidt-Nave C, Stepan J, Todd C, Weber K, Woolf AD, O'Neill TW. Incidence of limb fracture across Europe: results from the European Prospective Osteoporosis Study (EPOS). Osteoporos Int 2002; 13:565-71.

4 Finigan J, Greenfield DM, Blumsohn A, Hannon RA, Peel NF, Jiang G, Eastell R. Risk factors for vertebral and nonvertebral fracture over 10 years: a population-based study in women. J Bone Miner Res 2008; 23:75-85.

5 Peters MJ, Nielen MM, Raterman HG, Verheij RA, Schellevis FG, Nurmohamed MT. Increased cardiovascular disease in patients with inflammatory arthritis in primary care: a cross-sectional observation. J Rheumatol 2009; 36:1866-8.

6 Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM, Cathey MA. The mortality of rheumatoid arthritis. Arthritis Rheum 1994; 37:481-94.

7 Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005; 4:130-6.

8 Symmons DP. Epidemiology of rheumatoid arthritis: determinants of onset, persistence and outcome. Best Pract Res Clin Rheumatol 2002; 16:707-22.

9 Aarden L, Ruuls SR, Wolbink G. Immunogenicity of anti-tumor necrosis factor antibodies-toward improved methods of anti-antibody measurement. Curr Opin Immunol 2008; 20:431-5.

10 Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. N Engl J Med 2004; 350:2167-79.

11 Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999; 354:1932-9.

12 Pols HA, Felsenberg D, Hanley DA, Stepán J, Muñoz-Torres M, Wilkin TJ, Qin-sheng G, Galich AM, Vandormael K, Yates AJ, Stych B. Multinational, placebo-controlled, randomized trial of the effects of alendronate on bone density and fracture risk in postmenopausal women with low bone mass: results of the FOSIT study. Fosamax International Trial Study Group. Osteoporos Int 1999; 9:461-8.

13 Bauer DC, Black D, Ensrud K, Thompson D, Hochberg M, Nevitt M, Musliner T, Freedholm D. Upper gastrointestinal tract safety profile of alendronate: the fracture intervention trial. Arch Intern Med 2000; 160:517-25.

14 Le GB, Soltner E, Charrier C et al. A combination of methotrexate and zoledronic acid prevents bone erosions and systemic bone mass loss in collagen induced arthritis. Arthritis Res Ther 2009; 11(6):R185.

15 Jarrett SJ, Conaghan PG, Sloan VS, Papanastasiou P, Ortmann CE, O'Connor PJ, Grainger AJ, Emery P. Preliminary evidence for a structural benefit of the new bisphosphonate zoledronic acid in early rheumatoid arthritis. Arthritis Rheum 2006; 54:1410-4.

16 Zwerina J, Hayer S, Tohidast-Akrad M, Bergmeister H, Redlich K, Feige U, Dunstan C, Kollias G, Steiner G, Smolen J, Schett G. Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. Arthritis Rheum 2004; 50:277-90.

17 Cohen SB, Dore RK, Lane NE, Ory PA, Peterfy CG, Sharp JT, van der Heijde D, Zhou L, Tsuji W, Newmark R; Denosumab Rheumatoid Arthritis Study Group. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. Arthritis Rheum 2008; 58:1299-309.

18 Eddleston A, Marenzana M, Moore AR, Stephens P, Muzylak M, Marshall D, Robinson MK. A short treatment with an antibody to sclerostin can inhibit bone loss in an ongoing model of colitis. J Bone Miner Res 2009; 24:1662-71.

19 de Vries MK, Brouwer E, van der Horst-Bruinsma IE, Spoorenberg A, van Denderen JC, Jamnitski A, Nurmohamed MT, Dijkmans BA, Aarden LA, Wolbink GJ. Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. Ann Rheum Dis 2009; 68:1787-8.

20 van Kuijk AW, de GM, Stapel SO, Dijkmans BA, Wolbink GJ, Tak PP. Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. Ann Rheum Dis 2010; 69:624-5.

21 Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, Dijkmans BA, Tak PP, Wolbink GJ. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. Ann Rheum Dis 2010; 69:817-21.

Chapter 10

Samenvatting

Reumatoïde artritis: biologicals en bot

In deel I van dit proefschrift worden resultaten gepresenteerd van onderzoek naar gegeneraliseerd botverlies (osteoporose) in patiënten met reumatoïde artritis (RA). In deel II worden diverse studies beschreven die wij verricht hebben in ons cohort van patiënten met reumatoïde artritis, die behandeld werden met moderne anti-reumatische therapie; TNF-blokkers, in ons geval infliximab.

DEEL I

Reumatoïde artritis is een ziekte die gepaard gaat met schade aan de gewrichten. Hierbij is er niet alleen verlies van bot dat het gewricht vormt, maar is er ook gegeneraliseerd botverlies (osteoporose). Dit verlies van bot lijkt niet alleen geassocieerd met de activiteit van de ziekte, maar ook bijvoorbeeld met de medicatie die gebruikt wordt om reumatoïde artritis te behandelen (prednison). In het eerste deel van dit proefschrift worden enkele onderzoeken beschreven naar dit gegeneraliseerde botverlies bij RA.

In **hoofdstuk 2a** worden de korte-termijneffecten (14 weken) van infliximab op bot-metabolisme in patiënten met RA beschreven. De mate van botaanmaak en botafbraak werd bepaald met behulp van markers in het bloed voor beide processen. De markers voor botaanmaak vertoonden een lichte stijging en de markers voor botafbraak lieten een aanzienlijke daling zien gedurende de 14 weken van behandeling. De daling in botafbraakmarkers was gecorreleerd met een afname van de ziekteactiviteit.

In **hoofdstuk 2b** zijn de gegevens te zien van het effect van 1 jaar behandeling met infliximab op de botmineraaldichtheid (BMD) van wervelkolom, heupen en handen en op de markers van botmetabolisme in een cohort van 102 patiënten met reumatoïde artritis. Deze studie toonde aan dat door behandeling met infliximab het gegeneraliseerde botverlies in de heupen en de lumbale wervelkolom kon worden voorkomen. Echter, het lokale botverlies in de handen kon niet worden voorkomen. Bij patiënten met een goede klinische respons op de behandeling met infliximab (volgens de EULAR responscriteria), was er een lichte toename in BMD van de heup en de wervelkolom, terwijl er bij patiënten met een matige klinische respons een daling van de BMD te zien was. Deze relatie tussen ziekteactiviteit en botverlies werd verder ondersteund door de correlatie tussen afname in ziekteactiviteit (DAS-28 en CRP) en een daling van een marker voor botafbraak (ß-CTX). Tevens daalde de concentratie van een belangrijk eiwit (RANKL), dat een belangrijke rol speelt in de differentiatie, activering en overleving van botafbrekende cellen (osteoclasten) aanzienlijk gedurende de behandeling met infliximab. De blokker van dit eiwit (OPG) bleef echter stabiel in concentratie. Dit resulteerde in een gunstige verandering in de ratio van beide botafbraak regulerende eiwitten (RANKL/OPG).

Samengevat, behandeling met infliximab vermindert de botafbraak in patiënten met RA, wat leidt tot een vermindering van gegeneraliseerd botverlies.

In **hoofdstuk 3** worden veranderingen beschreven in de BMD van de wervelkolom, heup en handen en het optreden van fracturen gedurende 5 jaar bij patiënten met reumatoïde artritis. Dit onderzoek is uitgevoerd door de OSTRA-groep. De OSTRA-groep is een internationale onderzoeksgroep die bestaat uit reumatologen uit 3 Noord-Europese landen: **Os**lo (Noorwegen), **Tr**uro (Verenigd Koninkrijk) en **A**msterdam (Nederland). De OSTRA-groep onderzoekt verschillende aspecten van botmetabolisme bij patiënten met reumatische ziektes. Het OSTRA-cohort is een cohort dat bestaat uit 150 vrouwelijke RA-patiënten met gevorderde ziekte (> 5 jaar). Het belangrijkste resultaat van de eerder beschreven baselinestudie van dit cohort was, dat ernstige gewrichtsschade (gemeten door de Larsen-score) geassocieerd was met een lage BMD van de heup en een toename van wervelfracturen. In totaal werden 102 van deze 150 patiënten na 5 jaar opnieuw onderzocht en geïncludeerd in de follow-upstudie.

In **hoofdstuk 3a** volgt een beschrijving van fracturen die zich bij deze patiënten hebben voorgedaan tijdens de follow-upperiode. We ontdekten dat een groot aantal nieuwe wervel- en niet-wervelfracturen optrad gedurende die 5 jaar. Bij 18 van de 102 patiënten (17,6%) had zich een nieuwe, niet-wervelfractuur voorgedaan en bij 18 van 97 patiënten (18,5%) kon een nieuwe wervelfractuur worden geïdentificeerd op de foto van de wervelkolom. Dit komt neer op een jaarlijkse incidentie van 3,1 per 100 patiënten voor nieuwe niet-wervelfracturen en van 3,7 per 100 patiënten voor nieuwe wervelfracturen. Dit is duidelijk hoger dan de incidentie die doorgaans wordt gevonden in de algemene bevolking van vergelijkbare leeftijd en geslacht.

Bovendien vonden we een aanzienlijke daling van de BMD van de heup en de wervelkolom tijdens de 5 jaar follow-upperiode (**hoofdstuk 3b**). Er was een statistisch significante daling van 5,9% ($p<0,05$) van de BMD van de heup. Ook het verlies van BMD was groter dan verwacht mag worden in een populatie met vergelijkbare leeftijd en geslacht.

In **hoofdstuk 3c** wordt de verandering van BMD van de handen beschreven die we hebben gemeten met dual X-ray radiogrammatry (DXR). Er was een afname in de gemiddelde DXR-BMD van 6,7%. Hoge ziekteactiviteit aan het begin van de studie was een voorspeller voor verlies van BMD van de hand, gedurende de periode van 5 jaar.

Concluderend, er is bij RA-patiënten een toegenomen verlies van BMD met daarbij een verhoogde incidentie van wervel- en niet-wervelfracturen.

DEEL II

In het tweede deel van het proefschrift wordt een aantal studies gepresenteerd, die wij hebben verricht in ons cohort van de met infliximab (TNF-blokker) behandelde RA-patiënten. Dit cohort werd opgestart in het Slotervaartziekenhuis en het VU medisch centrum in 2001, kort na de introductie van infliximab in Nederland. Alle patiënten die behandeld werden met infliximab, werden geïncludeerd in dit cohort. Ziekteactiviteit en bijwerkingen werden structureel bijgehouden, bloedmonsters werden afgenomen bij elk bezoek en röntgenfoto's van handen en voeten werden jaarlijks verricht.

Patiënten met RA hebben een verhoogde mortaliteit in vergelijking met de algemene bevolking. Sterfte ten gevolge van hart- en vaatziekten (HVZ) is de belangrijkste oorzaak van de toegenomen sterfte bij patiënten met RA. Deze hoge incidentie van cardiovasculaire mortaliteit bij patiënten met RA lijkt voornamelijk het gevolg van versnelde atheroclerose. Er is tevens bewijs dat de ontsteking bij RA is geassocieerd met een verslechtering van het lipidenprofiel. In **hoofdstuk 4a** worden gegevens gepresenteerd van de korte-termijneffecten van de behandeling met infliximab op het lipidenprofiel in patiënten met RA. De behandeling met infliximab leidde tot een statistisch significante toename van zowel het totaal-cholesterol als het HDL-cholesterol. Deze veranderingen waren omgekeerd evenredig met de verandering in de ziekteactiviteit. Echter, de atherogene index (verhouding tussen totaal-cholesterol en HDL-cholesterol), een belangrijke prognostische indicator voor toekomstige hart- en vaatziekten, bleef onveranderd.

In **hoofdstuk 4b** worden de effecten op de langere termijn (1 jaar) van infliximab op het lipidenprofiel in RA-patiënten gepresenteerd. In deze studie zagen we een aanzienlijke toename van het totaal-cholesterol en HDL-cholesterol in de eerste 6 weken van behandeling met infliximab, waarna deze vervolgens geleidelijk terugzakten tot de uitgangswaarde. Uit analyses bleek, dat er een tegenovergesteld effect was op de lipidenspiegels tussen ziekteactiviteit en prednisondosis. Een vermindering van de ziekteactiviteit was geassocieerd met een toename in totaal-cholesterol en HDL-cholesterol. Een afname van prednison was echter geassocieerd met een daling van totaal-cholesterol en HDL-cholesterol. Het lijkt er dus op dat de initiële effecten van infliximab op het lipidenprofiel door een vermindering van de ziekteactiviteit teniet worden gedaan door een verlaging van de prednisondosis omdat de ziekteactiviteit is afgenomen.

Samenvattend kan worden gesteld dat er een duidelijk effect van infliximab op het lipidenprofiel is door vermindering van de ziekteactiviteit. Deze verandering leidt echter niet tot een minder atherogeen lipidenprofiel.

Eén van de kenmerken van RA is de aanwezigheid van auto-antilichamen; IgM-reumafactor en anti-CPP. Anti-CCP antistoffen zijn antistoffen gericht tegen gecitrulineerde eiwitten en zijn zeer specifiek voor patiënten met RA. Van anti-CCP antistoffen wordt gedacht dat ze mogelijk ook een rol spelen bij het ontstaan van RA, mede omdat deze antistoffen al voorkomen bij patiënten voordat de ziekte zich openbaart. In **hoofdstuk 5** wordt aangetoond dat in ons cohort van RA-patiënten die gedurende een jaar behandeld werden met infliximab, zowel de IgM-RF- als de anti-CCP-titer aanzienlijk gedaald waren. Ook antistoffen tegen gedeimineerd fibrinogeen (ACF), een specifiek gecitrullineerde peptide die in het bijzonder gevonden wordt in ontstoken gewrichten van RA-patiënten, daalden aanzienlijk. In termen van percentages daalde de IgM-RF-titer met 64%, terwijl de anti-CCP- en ACF-niveaus werden verminderd met ongeveer 25%. ACF-niveaus toonden een grotere daling bij patiënten met vroege ziekte (ziekte van korte duur). Dit zou kunnen duiden op een mogelijk "window of opportunity" voor beïnvloeding van het ontstaan van RA.

Behandeling met infliximab is zeer effectief bij veel RA-patiënten. Echter, bij sommige patiënten treedt er een verlies van werkzaamheid op na een periode van succesvolle behandeling. Infliximab is een medicament dat niet volledig lichaamseigen is (deels mens, deels muis); waardoor er antistoffen tegen infliximab kunnen ontstaan. Door deze antistoffen zou er verlies van werkzaamheid kunnen optreden en zouden er bijwerkingen kunnen ontstaan. Uit de resultaten gepresenteerd in **hoofdstuk 6** blijkt dat antilichamen tegen infliximab konden worden aangetoond in het bloed van 22 patiënten (43%) behandeld met infliximab. Patiënten met anti-infliximab antistoffen werden significant vaker gevonden in de groep die niet goed reageerde op infliximab in vergelijking met de groep patiënten die wel een goede reactie vertoonde na 14 weken. Opmerkelijk is, dat bij alle patiënten met een allergische reactie (n=3) anti-infliximab antistoffen konden worden aangetoond.

Anti-infliximab antistoffen kunnen dus leiden tot verlies van effectiviteit en kunnen allergische reacties veroorzaken bij het gebruik van infliximab.

In de registratiestudies is infliximab naast een effectieve ook een veilige behandeling voor patiënten met RA. Deze gegevens kunnen echter niet altijd vertaald worden naar de dagelijkse praktijk vanwege de selectie van relatief gezonde patiënten in gerandomiseerde klinische studies. Daarom hebben wij alle infecties onderzocht in ons cohort waarin het gaat om patiënten uit de dagelijkse praktijk die behandeld worden met infliximab (**hoofdstuk 7**). Veel patiënten maakten minimaal één infectie door (43–57%, afhankelijk van de gebruikte definitie); meestal waren dit infecties van de bovenste luchtwegen en de urinewegen. De incidentie van ernstige infecties was 0,08 per patiënt/jaar. Deze getallen zijn hoger dan voor de algemene populatie, maar vergelijkbaar met de infectiefrequenties die gevonden werden in gerandomiseerde

studies naar infliximab bij patiënten met een reumatische aandoening. Hieruit blijkt dat infliximab relatief veilig is in de dagelijkse klinische praktijk.

Gastrolintestinale klachten zijn de meest voorkomende bijwerkingen van orale bisfosfonaten zoals alendronaat en risedronaat. Het bisfosfonaat pamidronaat kan echter ook worden toegediend middels intraveneuze infusie. Daarom wordt er gebruik gemaakt van pamidronaat voor patiënten die orale bisfosfonaten niet kunnen verdragen. In **hoofdstuk 8** worden de veranderingen in BMD gedurende 1 jaar behandeling met intraveneus pamidronaat (60 mg elke 3 maanden) beschreven bij patiënten die orale bisfosfonaten niet tolereren, in vergelijking met patiënten die ze wel verdragen en die worden behandeld met alendronaat. Deze studie toont aan, dat de behandeling met intraveneus pamidronaat net zo effectief is als orale behandeling met alendronaat, gemeten door het effect op de BMD.

Dankwoord
(Acknowledgements)

Dit proefschrift zou niet tot stand gekomen zijn zonder de directe of indirecte hulp van velen. Ik wil dan ook alle medewerkers van het VUmc, Jan van Breemen Instituut (thans READE) en het Slotervaartziekenhuis en vele anderen met wie ik samen heb gewerkt tijdens mijn promotieperiode hartelijk bedanken. Een aantal wil ik graag persoonlijk noemen.

Promotoren en copromotoren

Professor Lems, beste Willem, bedankt voor je aanhoudende enthousiasme over en inzet voor mijn promotie. Het duurde soms wat langer dan gepland voordat de stukken er waren, maar je bleef altijd stimulerend en positief. Tevens bedankt voor alle gezellige momenten, waarvan vooral de autoreis naar Kopenhagen mooie verhalen heeft opgeleverd.

Professor Dijkmans, hartelijk dank voor de mogelijkheid om aan uw afdeling promotieonderzoek te kunnen doen en voor de goede samenwerking. Tevens dank voor het altijd deskundige en snelle commentaar op mijn artikelen.

Dr. Wolbink, beste Gertjan, door jouw enthousiasme voor onderzoek ben ik aan deze promotie begonnen. Bedankt hiervoor en voor je altijd kritische commentaar en je unieke invalshoeken.

Dr. Voskuyl, beste Alexandre, bedankt voor je begeleiding bij dit proefschrift. Vooral dank voor je stimulerende commentaar bij de laatste loodjes van dit traject.

Leescommissie

Prof.dr. T.W.J Huizinga, prof.dr. P.P. Tak, prof.dr. P.P.M. Geusens, prof.dr. P.T.A. Lips, prof.dr. M.H.H. Kramer en prof.dr. Y.M. Smulders, hartelijk dank voor de genomen tijd ter beoordeling van het proefschrift.

Paranimfen

Beste Guus en Marvin, fijn dat jullie vandaag mijn paranimfen willen zijn en bedankt voor onze jarenlange vriendschap.

Afdeling 9b Slotervaartziekenhuis

Beste Ellen, Frank en Loes, bedankt voor de hulp bij het verzamelen van de data van de RA-patiënten die op de dagbehandeling behandeld werden met infliximab.

Kamer 4A64 VUmc

Beste Gerrit, Marleen, Mariëtte, Ruud, Joost, Ernst, Irene en Mirjam, bedankt voor de gezellige en prettige werkomgeving op de afdeling reumatologie. Tevens bedankt voor de goede gesprekken over onderzoek doen, statistiek, resultaten, maar ook over niet-wetenschapgerelateerde onderwerpen. Gezien het feit dat jullie proefschriften bijna klaar dan wel klaar zijn, hebben jullie gelukkig niet al te veel last ondervonden van mijn geklets en van de incidentele "zuurkool met vette jus".

Kamer B9 Jan van Breemen Instituut

Beste Vokko, Mark en Marga, bedankt voor de goede werksfeer en gezelligheid in het Jan van Breemen. Ondanks de ruimte van 4 bij 4 meter met 4 computers zaten we elkaar nooit in de weg en zijn mijn wetenschappelijke inzicht en kennis hier snel gegroeid. Vooral ook erg bedankt voor de fijne vriendschap die wij hadden in deze periode zowel tijdens als na het werk. Aan onze trip naar de EULAR in een oververhit (40+ °C) Lissabon heb ik een hoop goede herinneringen overgehouden.

Ook de latere onderzoekscollega's van het JBI, Wouter, Izhar, Jenny en Mike, bedankt voor de goede discussies en samenwerking.

Ook de collega's van het Laboratorium, Rob en Margret, bedankt voor jullie hulp bij de botmarkerartikelen en het verzamelen van spijtmateriaal. Dr. Nurmohamed, beste Mike, bedankt voor je inzet voor mijn proefschrift en het gebruik van je "research koelkast" voor een koud biertje op de vrijdagmiddagborrel.

The OSTRA Group

Dear Tore, Tony, Glenn, Espen, Pernille, Thil and Mari, thank you for the always friendly and inspiring meetings and the always very useful and to the point comments on the collaborative manuscripts. A special thanks to Espen for his hospitality during my trips to Oslo.

Bij het schrijven van dit boekje was er niet alleen hulp van "de professionals", maar ook steun en hulp van thuis. Hiervoor veel dank en liefde, Marike, Berend en David.

About the author

CURRICULUM VITAE

Marijn Vis werd geboren op 13 december 1975 te Utrecht. In 1994 behaalde hij zijn VWO-diploma aan het dr. F.H. de Bruijne Lyceum in Utrecht. Van 1994 tot 2001 studeerde hij geneeskunde aan de Vrije Universiteit in Amsterdam. Na het behalen van het artsexamen, begon hij als arts-onderzoeker bij de vakgroep reumatologie aan het VU medisch centrum onder leiding van professor B.A.C. Dijkmans en professor W.F. Lems. Tijdens deze periode verrichte hij werkzaamheden aan de afdelingen reumatologie van het Slotervaartziekenhuis, het Jan van Breemen Instituut en het VUmc. Voor zijn werk aan het artikel: *"High incidence of vertebral and non-vertebral fractures in the OSTRA-cohort study: a 5 year follow-up study in postmenopausal women with Rheumatoid Arthritis"*, ontving hij in 2010 de ECTS young investigator award.

In 2006 startte hij met zijn opleiding tot reumatoloog als art-assistent interne geneeskunde in het Slotervaartziekenhuis (opleider: dr. D. Brandjes). In Januari 2009 begon hij aan het reumatologiedeel van de opleiding in het Jan van Breemen Instituut (opleider: dr. D. van Schaardenburg). Sinds 2010 is hij werkzaam als arts-assistent reumatologie in het VU medisch centrum (opleider: dr. A.E. Voskuyl).

Marijn Vis is getrouwd met Marike van Hengel. Zij hebben twee zoons, Berend en David.

PUBLICATIONS

Vis M, Haavardsholm EA, Bøyesen P, Haugeberg G, Uhlig T, Hoff M, Woolf A, Dijkmans B, Lems WF, Kvien TK. High incidence of vertebral and non-vertebral fractures in the OSTRA cohort study: a 5-year follow-up study in postmenopausal women with rheumatoid arthritis. Osteoporos Int 2011; 13.

Hoff M, Bøyesen P, Haugeberg G, Vis M, Woolf AD, Havaardsholm EA, Dijkmans BA, Kvien TK, Uhlig T, Lems WF. High disease activity is a predictor of cortical hand bone loss in post-menopausal patients with established rheumatoid arthritis: a 5-year multicentre longitudinal study. Rheumatology (Oxford) 2010; 49:1676-82.

Eekman DA, Vis M, Bultink IE, Kuik DJ, Voskuyl AE, Dijkmans BA, Lems WF. Stable bone mineral density in lumbar spine and hip in contrast to bone loss in the hands during long-term treatment with infliximab in patients with rheumatoid arthritis. Ann Rheum Dis 2011; 70:389-90.

Eekman DA, Vis M, Bultink IE, Derikx HJ, Dijkmans BA, Lems WF. Treatment with intravenous pamidronate is a good alternative in case of gastrointestinal side effects or contraindications for oral bisphosphonates. BMC Musculoskelet Disord 2009; 10:86.

Bos WH, Bartelds GM, Vis M, van der Horst AR, Wolbink GJ, van de Stadt RJ, van Schaardenburg D, Dijkmans BA, Lems WF, Nurmohamed MT, Aarden L, Hamann D. Preferential decrease in IgG4 anti-citrullinated protein antibodies during treatment with tumour necrosis factor blocking agents in patients with rheumatoid arthritis. Ann Rheum Dis 2009; 68:558-63.

Vis M, Bos WH, Wolbink G, Voskuyl AE, Twisk JW, Van de Stadt R, Hamann D, Dijkmans BA, Lems WF. IgM-rheumatoid factor, anti-cyclic citrullinated peptide, and anti-citrullinated human fibrinogen antibodies decrease during treatment with the tumor necrosis factor blocker infliximab in patients with rheumatoid arthritis. J Rheumatol 2008; 35:425-8.

Peters MJ, Vis M, van Halm VP, Wolbink GJ, Voskuyl AE, Lems WF, Dijkmans BA, Twisk JW, de Koning MH, van de Stadt RJ, Nurmohamed MT. Changes in lipid profile during infliximab and corticosteroid treatment in rheumatoid arthritis. Ann Rheum Dis 2007; 66:958-61.

Vis M, Havaardsholm EA, Haugeberg G, Uhlig T, Voskuyl AE, van de Stadt RJ, Dijkmans BA, Woolf AD, Kvien TK, Lems WF. Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFkappaB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. Ann Rheum Dis 2006; 65:1495-9.

Wolbink GJ, Vis M, Lems WF, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54:711-5.

Vis M, Bultink IE, Dijkmans BA, Lems WF. The effect of intravenous pamidronate versus oral alendronate on bone mineral density in patients with osteoporosis. Osteoporos Int 2005;16:1432-5.

Neven N, Vis M, Voskuyl AE, Wolbink GJ, Nurmohamed MT, Dijkmans BA, Lems WF. Adverse events in patients with rheumatoid arthritis treated with infliximab in daily clinical practice. Ann Rheum Dis 2005; 64:645-6.

Vis M, Nurmohamed MT, Wolbink G, Voskuyl AE, de Koning M, van de Stadt R, Twisk JW, Dijkmans BA, Lems WF. Short term effects of infliximab on the lipid profile in patients with rheumatoid arthritis. J Rheumatol 2005; 32:252-5.

Vis M, Voskuyl AE, Wolbink GJ, Dijkmans BA, Lems WF; OSTRA Study Group. Bone mineral density in patients with rheumatoid arthritis treated with infliximab. Ann Rheum Dis 2005; 64(2):336-7.

Vis M, Wolbink GJ, Lodder MC, Kostense PJ, van de Stadt RJ, de Koning MH, Dijkmans BA, Lems WF. Early changes in bone metabolism in rheumatoid arthritis patients treated with infliximab. Arthritis Rheum 2003; 48:2996-7.