VU VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Preclinical rheumatoid arthritis

Nielen, M.M.J.

2009

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Nielen, M. M. J. (2009). *Preclinical rheumatoid arthritis*. [PhD-Thesis - Research and graduation internal, S.l.]. s.n.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 14. Feb. 2025

Chapter 7

Bone metabolism is altered in preclinical rheumatoid arthritis

MMJ Nielen[1], WF Lems[1,2], JWR Twisk[2], HW Reesink[3], RJ van de Stadt[1], IE van der Horst-Bruinsma[2], MHMT de Koning[1], MR Habibuw[3], BAC Dijkmans[2,1] and D van Schaardenburg[1, 2]

[1] *Jan van Breemen Institute, Amsterdam, The Netherlands*
[2] *VU University Medical Centre, Amsterdam*
[3] *Sanquin Blood Bank Northwest Region, Amsterdam*

Submitted

Abstract

Objective: Increased levels of autoantibodies and inflammation markers occur years before onset of symptoms of rheumatoid arthritis (RA). The present study investigates whether presence of autoimmunity and inflammation in preclinical RA is accompanied by alterations in bone metabolism.

Methods: Seventy-nine patients had donated blood before onset of RA. From each patient 3 samples were selected: 1, 2 and 5 years or longer before onset of symptoms, respectively, together with one control sample. The following markers were measured: 1) markers for bone formation: osteocalcin (OC) and N-terminal propeptide of type I collagen (P1NP), 2) a marker of bone resorption: β-C-telopeptide (β-CTX), and 3) regulators of osteoclast activity: receptor activator of NFκB ligand (RANKL) and osteoprotegerin (OPG). The mean values at the specified time points of the different markers in the patient and control groups were compared with random coefficient analysis. Variables differing statistically significantly from controls were then tested for their possible association with future radiographic progression using linear regression analysis.

Results: Correcting for age, gender, time of blood donation, autoantibodies and inflammation, preclinical RA patients had increased mean levels of P1NP and OPG compared with the control group. Preclinical levels of P1NP and OPG were negatively associated with radiographic progression after the onset of the symptoms of RA, but these associations were not statistically significant.

Conclusion: The asymptomatic phase of RA is characterized not only by autoimmunity and increased inflammation, but also by a parallel alteration of bone metabolism.

Introduction

The chronic inflammation of the joints in rheumatoid arthritis (RA) may lead to the destruction of cartilage and bone. Thirty percent of patients with newly diagnosed RA already have joint damage that is visible on radiographs after a median symptom duration of only three months [1]. Before the era of modern combination therapies, this figure increased to 75% two years after the onset of the disease [2]. Radiographic damage is associated with a loss of functional capacity in a later stage of the disease [3, 4]. Since joint damage is irreversible but potentially avoidable by modern treatment, it is important to diagnose and treat RA early, preferably before joint damage occurs [5, 6].

The development of radiographic damage in RA can be predicted by measurements of markers and regulators of bone metabolism in blood and urine. Measurements of bone formation in RA patients have produced varying results, whereas measurements of bone resorption in these patients mostly show increased values. The activity of bone formation by the osteoblast can be measured among others by osteocalcin (OC) and by the N-terminal telopeptide of type I procollagen (P1NP). RA patients have significantly lower OC levels in comparison with healthy controls [7], however, differences in OC levels between RA patients with and without radiographic progression could not be found [7, 8]. Contrarily, elevated P1NP levels were found in RA patients compared with healthy controls [9]. The association between P1NP levels and radiographic progression has not yet been studied in RA. The activity of bone degradation by the osteoclast can be measured by, among others, the C-terminal crosslink of type I collagen (β-CTX), a collagen C-telopeptide breakdown product. RA patients have significantly higher urine and serum β-CTX levels compared with healthy controls [7, 9]. Serum β -CTX is associated with radiographic damage in early arthritis [7, 8] and higher β-CTX concentrations were found in erosive arthritis patients in comparison with non-erosive arthritis patients [8]. In addition, urine β-CTX levels are significantly correlated with future radiographic progression [10-12].

The activation of the osteoclast is regulated by the Receptor Activator of Nuclear Factor Kappa B (NFκB) ligand (RANKL) and osteoprotegerin (OPG), in combination with Receptor Activator of NFκB (RANK). RANKL is an osteoclast-activating cytokine that is produced by osteoblasts and activated T-cells, whereas OPG, a soluble receptor produced by a variety of tissues including the cardiovascular system, lung, kidney, intestine, and bone, as well as hematopoietic and immune cells, prevents osteoclast activation. RA patients have higher serum levels of both OPG and RANKL in comparison with matched healthy controls [13]. In the COBRA-study, high RANKL and low OPG levels were associated with an increased risk of radiographic damage in early arthritis patients [14].
In a previous study we found increased levels of rheumatoid factor (IgM-RF) and/or antibodies against cyclic citrullinated peptides (anti-CCP) in one half of blood donors, starting at median 5 years before they developed the first symptoms of RA [15]. We also demonstrated increased levels of acute phase reactants in these patients years before the onset of symptoms, regardless of their autoantibody status [16, 17]. The present study was performed to investigate whether this preclinical subtle increase of inflammation has an influence on markers of bone metabolism. Therefore, we tested whether concentrations of markers of bone metabolism and regulators of osteoclast activity in preclinical RA patients differ from healthy controls at three time points in the preclinical phase of RA. In case of any differences between preclinical RA patients and controls, we investigated whether these markers and/or regulators were associated with radiographic damage after the onset of the symptoms of RA.

Patients and methods

Patients

Since 1984, the Sanquin Blood Bank North West Region in Amsterdam, The Netherlands, has stored 1-ml aliquots of serum from donated blood at -30˚C. From 5000 patients registered with RA at the Jan van Breemen Institute, 79 patients were identified who had donated blood, in general 2-4 times per year, before the onset of RA, as described previously [15] . All blood donations from a particular RA patient in the period 1984-1999 were traced. For each RA sample, 1 control sample was selected, matched for gender, age, and time of blood donation to ensure identical storage conditions. Also, the most recent available

radiographs of hand and feet of the 79 RA patients were collected. The study was approved by the local Institutional Review Board.

Procedures

In previous studies we used all patient samples (median 13 samples per patient) and for each patient sample a matched control sample [15-17]. In the present study, from each later RA patient three serum samples were selected, if available: at 1 year, 2 years and 5 years or longer before the start of the symptoms, respectively, together with the corresponding matched control sample. The percentage of controls positive for IgM-RF and anti-CCP and the median CRP concentrations of the controls in this study were comparable with the findings in the previous studies [15-17].

The following markers were measured: 1) two markers for bone formation: OC and P1NP, 2) a marker of bone resorption: β-CTX, and 3) two counteracting regulators of osteoclast activity: RANKL and OPG. All markers were measured using commercial assays according to the instructions of the manufacturers. OC (N-mid), P1NP and β-CTX were measured with an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Total RANKL and OPG were measured with appropriate ELISA kits from Immundiagnostik, Bensheim, Germany. IgM-RF, anti-CCP, secretory phospholipase A_2 (sPLA2) and C-reactive protein (CRP) were measured in previous studies [15-17].

All available radiographs were scored according to the Sharp/van der Heijde method [17] by an experienced rheumatologist (DvS), who was blinded for all preclinical test results.

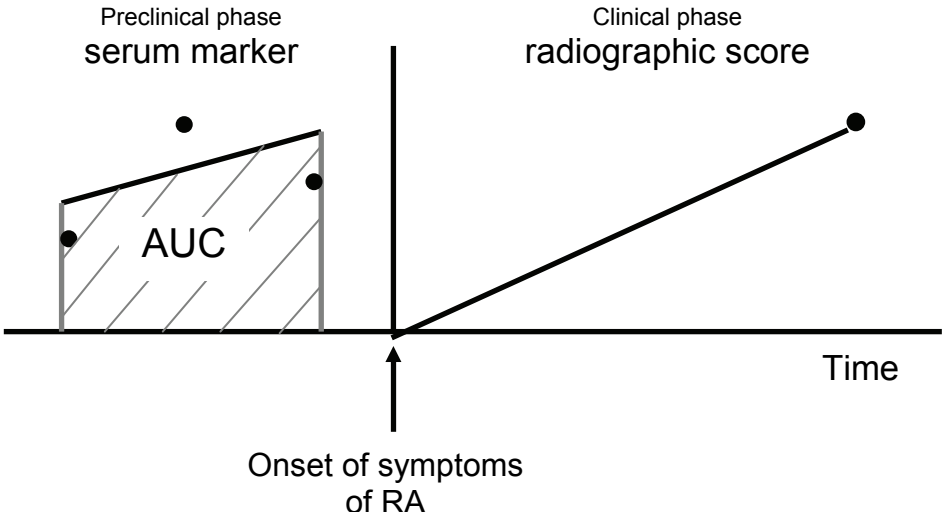
Analysis

In the first analysis, only preclinical RA patients with samples at all three time points were selected with matched controls. At all time points the mean concentrations of OC, P1NP, β-CTX and OPG of the group of preclinical RA patients were compared with those of the group of healthy controls by using independent t-tests. Median RANKL levels were compared between the groups of patients and controls with Mann-Whitney U tests.

The first analysis was performed with only the patients who had samples available at all three time points and was not corrected for potential confounding factors, i.e. gender, age, the presence of autoantibodies and levels of markers of inflammation. Therefore, comparable analyses were performed with all patient samples in the period of 0-6 years before the onset of symptoms and in matched control samples. In this second analysis, differences in OC, P1NP, β-CTX, OPG and RANKL over time in preclinical RA patients were compared with controls with

random coefficient analysis. This method was used to correct for the different number of samples per patient and the variation in the time of the blood donation [18]. Samples obtained six years or longer before the onset of symptoms were not used because of their small number. All regression models were corrected for age, gender, time of donation, autoantibodies (IgM-RF and anti-CCP) and markers of inflammation (CRP and sPLA2). Because the distribution of RANKL concentrations was skewed to the right, the natural log of RANKL concentrations was used in the analysis.

Figure 7.1: Graph of the analysis of the association per individual between preclinical serum markers and radiographic scores



AUC: area under the curve

If a bone marker or a regulator of osteoclast activity differed between preclinical RA patients and healthy controls in the random coefficient analysis, this parameter was associated with future radiographic progression within the group of preclinical RA patients. For each patient, individual regression lines were calculated for the specific markers and/or regulators of bone metabolism. These individual regression lines were used to calculate the mean individual area under the curve (AUC) per year (see figure 7.1). Not for all patients it was possible to calculate an individual regression line. When a particular parameter was not

measured in an individual, the mean AUC per year of the whole group was used. When only one value of a parameter was available, the mean slope of the whole group was used to measure the individual mean AUC per year. The mean AUC per year of the bone markers and regulators were associated with the mean radiographic progression per year (i.e. the most recent Sharp/ van der Heijde score divided by the number of years with symptoms of the patient at the time of this radiological measurement) with linear regression analysis. The association was also corrected for age at the start of the symptoms of RA, gender and the presence of autoantibodies in the preclinical phase.
Linear regression analyses were carried out with SPSS 14.0. Random coefficient analyses were performed with MLwiN (Multilevel Models Project, Institute of Education, University of London, London, UK).

Results

Patient characteristics
Seventy-nine patients (62% female; mean age at onset of the symptoms 51 years) who had been blood donor before the onset of RA were identified. A median of 13 serum samples per patient (range 1–51) was available; the median time between the first donation and the onset of the symptoms was 7.5 years (range 0.1–14.5 years). In total, 1078 patient sera and 1071 matched control sera were identified. From each patient 3 samples were selected: at 1 year, 2 years and 5 years or longer before the start of the symptoms, respectively, together with one matched control donor sample. In this study, 192 patient sera and 191 matched control sera were tested. For 32 patients it was not possible to select a sample at all three time points; 18 patients had two samples and 14 patients had one sample available. For one patient sample there was no matched control sample.
Radiographs of hands and feet were available for 71 out of 79 patients (90%). The mean time between the start of the symptoms and the radiographs was 7.2 years. Median Sharp/van der Heijde score at that time point was 16 (IQ-range 5-49).

Bone markers and regulators of osteoclast activity
Forty-six out of 79 (58%) preclinical RA patients had samples at all three time points. The mean bone marker levels of preclinical RA patients and matched controls at one, two and five years before the start of the symptoms of RA are

shown in table 7.1. OPG was increased in preclinical RA patients compared with controls at all three time points (P<0.001). In preclinical RA patients at one year and two years before the onset of symptoms of RA, P1NP and RANKL levels were increased, but these difference were not statistically significant (for P1NP p=0.12 and p=0.07, and for RANKL p=0.14 and p=0.09, respectively). β-CTX was increased in preclinical RA patients one year before the start of symptoms, but this difference was also not statistically significant (p=0.15).
In the next analysis, all patient samples and matched controls in the time period of 0-6 before the start of the symptoms of RA were used to correct the differences between preclinical RA patients and controls for potential confounding variables in a multivariate regression analysis. The regression models for the bone markers and the regulators of osteoclast activity are shown in table 7.2. After correction for age, gender, time of blood donation, autoantibodies and markers of inflammation, the group of preclinical RA patients had statistitically significantly increased mean levels of P1NP and OPG compared with the control group (an increase of 5.0 ng/ml and 4.1 pmol/l for P1NP and OPG, respectively). Differences in median RANKL and mean β-CTX levels between the patient and control groups in the first univariate analysis (table 7.1) were no longer found after correction for age, gender, autoantibodies and inflammation.

Table 7.1: Mean bone marker levels of preclinical RA patients and matched controls at one, two and five years before the start of the symptoms of RA

	Patients		Controls		p-value
1 year before onset symptoms					
P1NP (ng/ml)	46.5	(19.1)	39.6	(22.1)	0.12
OC (ng/ml)	19.9	(7.9)	18.3	(8.3)	0.36
β-CTX (ng/ml)	0.13	(0.08)	0.11	(0.05)	0.15
OPG (pmol/l)	10.0	(5.5)	5.2	(1.5)	0.00
RANKL (pmol/l)*	653.5	(401.0-1582.0)	491.5	(330.3-1179.0)	0.14
2 years before onset symptoms					
P1NP (ng/ml)	49.4	(22.0)	40.6	(23.0)	0.07
OC (ng/ml)	20.6	(8.7)	18.8	(7.9)	0.30
β-CTX (ng/ml)	0.10	(0.06)	0.11	(0.07)	0.75
OPG (pmol/l)	8.6	(2.9)	4.5	(1.3)	0.00
RANKL (pmol/l)*	645.5	(387.3-1617.0)	437.0	(318.8-1064.8)	0.09
5 years before onset symptoms					
P1NP (ng/ml)	46.3	(19.9)	43.3	(23.2)	0.51
OC (ng/ml)	18.8	(6.4)	18.5	(8.0)	0.86
β-CTX (ng/ml)	0.11	(0.07)	0.10	(0.05)	0.20
OPG (pmol/l)	7.6	(2.5)	4.6	(1.3)	0.00
RANKL (pmol/l)*	619	(393.3-1544.8)	570.5	(347.3-1098.5)	0.35

P1NP = N-terminal telepeptide of type I procollagen, OC = Osteocalcin, β-CTX = C-terminal crosslink of type I collagen, OPG = Osteoprotegerin, RANKL = Receptor activator of NFκB ligand.
* Median and inter-quartile range

Table 7.2: Association between bone markers and the presence of preclinical RA, corrected for time of donation, age, gender, presence of autoantibodies and inflammation

	B (standard error)									
	P1NP		OC		CTX		OPG		Ln RANKL	
Preclinical RA versus controls	**5.02**	**(2.00)**	1.06	(0.74)	0.0081	(0.0067)	**4.10**	**(0.33)**	-0.02	(0.10)
Time of donation (years)	0.40	(0.60)	0.12	(0.22)	-0.0001	(0.0020)	-0.18	(0.09)	0.00	(0.03)
Age (years)	-0.07	(0.13)	0.09	(0.05)	0.0001	(0.0004)	**0.05**	**(0.02)**	0.00	(0.01)
Gender (female vs male)	2.64	(2.98)	1.44	(1.07)	-0.0037	(0.0094)	0.32	(0.35)	-0.06	(0.13)
sPLA2 (ng / ml)	0.32	(0.24)	0.15	(0.09)	-0.0004	(0.0008)	0.00	(0.04)	**0.03**	**(0.01)**
CRP (mg / l)	**-0.65**	**(0.25)**	**-0.31**	**(0.09)**	-0.0009	(0.0008)	0.06	(0.04)	-0.01	(0.01)
IgM-RF (IU / ml)	-0.05	(0.03)	-0.01	(0.01)	-0.0001	(0.0001)	-0.01	(0.00)	**0.01**	**(0.00)**
Anti-CCP (AU / ml)	0.00	(0.00)	0.00	(0.00)	0.0000	(0.0000)	0.00	(0.00)	0.00	(0.00)
Intercept	40.52	(8.95)	11.74	(3.21)	0.1112	(0.1112)	2.20	(1.05)	6.24	(0.40)

Statistically significant B's are in bold; The models are calculated with random coefficient analysis.
P1NP = N-terminal telepeptide of type I procollagen, OC = Osteocalcin, β-CTX = C-terminal crosslink of type I collagen, OPG = Osteoprotegerin
RANKL = Receptor activator of NFκB ligand, sPLA2 = secretory phospholipase A_2, CRP = C-reactive protein, IgM-RF = rheumatoid factor, Anti-CCP = antibodies against cyclic citrullinated peptides.

Association between preclinical serum markers and radiographic progression

If a bone marker or a regulator of osteoclast activity differed between preclinical RA patients and healthy controls in the multivariate analysis, this parameter was associated with future radiographic progression within the group of preclinical RA patients. Based on the previous analysis, P1NP and OPG are potential predictors of radiographic progression in preclinical RA patients, since the mean levels of these two markers were increased in preclinical RA patients. For these two markers, the mean individual AUC per year of the preclinical serum markers was associated with the mean progression per year of the Sharp/van der Heijde score. After correction for age, gender and the presence of preclinical autoantibodies, preclinical levels of P1NP and OPG were negatively associated with radiographic progression after the onset of the symptoms of RA (B=-0,60 (p=0.12) and B=-0,09 (p=0.16) for P1NP and OPG, respectively), but these differences were not statistically significant (Table 7.3).

Table 7.3: Association of P1NP and OPG and future radiographic progression in preclinical RA, corrected for age, gender and preclinical presence of autoantibodies

	B (standard error)			
	P1NP		OPG	
AUC rediographic damage (Sharp / van der Heijde score)	-0,60	(0,38)	-0,09	(0,07)
Gender (female versus male)	**9,67**	**(4,64)**	0,39	(0,81)
Age at start of the symptoms of RA (years)	0,01	(0,22)	0,06	(0,04)
Preclinical IgM-RF positivity	1,79	(5,72)	-0,44	(0,99)
Preclinical anti-CCP positivity	0,76	(4,85)	-0,44	(0,84)
Intercept	38,25	(13,25)	6,84	(2,30)

Statistically significant B's are in bold; The models are calculated with linear regression analyses.
P1NP = N-terminal telepeptide of type I procollagen, OPG = Osteoprotegerin, AUC = area under the curve, IgM-RF = rheumatoid factor, Anti-CCP = antibodies against cyclic citrullinated peptides.

Not all patients had two or more measurements for each parameter, which is necessary to calculate an individual regression line. Therefore, we used the mean slope of the whole group for these patients to calculate the individual mean AUC per year. This method was compared with two other methods: 1) only using the AUC of the patients who had two or three measurements of the specific parameter, and 2) using a slope of zero (beta = 0). The calculated B's did not differ between the three statistical methods (data not shown) and therefore we used the described method to increase the power of the analysis since more patients could be included.

Discussion

In this group of preclinical RA patients a number of abnormalities were found in serum markers of bone metabolism and osteoclast regulation. OPG was increased compared with controls at one, two and five years before the start of the symptoms of RA. Statistically non-significant findings were increased levels of P1NP and RANKL (at one year and two years before the onset of symptoms) and β-CTX (at one year before the start of symptoms). With multivariate analysis, correcting for age, gender, time of blood donation, autoantibodies and inflammation, preclinical RA patients proved to have increased levels of P1NP

and OPG compared with controls. Preclinical levels of P1NP and OPG were also negatively associated with radiographic progression after the onset of the symptoms of RA, but these differences were not statistically significant.
The levels of OC and β-CTX (indicating the activity of bone formation and degradation, respectively) in preclinical RA patients were similar to those found in healthy controls. This is in contrast to studies in RA patients with established and active disease, in which lower serum OC levels and higher urine and serum β-CTX levels were found compared with healthy controls [7, 9, 10, 19]. P1NP levels were increased in preclinical RA, which is in line with results in RA patients [9]. This suggests that significant differences in concentrations of markers of bone formation can be detected above a certain level of “disease activity” in the presymptomatic phase of RA. Also, elevated levels of OPG were found, which indicates an altered, probably increased, osteoclast activity in the preclinical phase.
We found a trend for a negative association between preclinical levels of P1NP and OPG and radiographic joint damage after the onset of the symptoms of RA. This suggests that a low level of bone formation as well as a low level of inhibition of osteoclast activation in the preclinical phase of RA is associated with more radiographic damage after the start of the symptoms of RA. In theory, it might be expected that preclinical inflammation would lead to increased preclinical bone degradation as a forerunner of radiographic damage in the clinical phase, indicating that bone resorption is upregulated by inflammatory processes. Instead of increased bone degradation, however, we found increased OPG. One possible explanation of the observed phenomena could be that the elevation of OPG is an - albeit failing - compensation mechanism for a slight and not measurable increase in bone degradation. In the clinical phase of early RA, one study found that high RANKL and low OPG levels are associated with an increased risk of radiographic damage, which is not in line with the results of the present study [14]. The possibility of bone damage occurring in the preclinical phase of RA is supported by the recent demonstration of erosive infiltration of joint margins in the asymptomatic phase of collagen induced arthritis, an animal model of RA [20].
The present study has some limitations. Differences in the mean levels of markers and/or regulators of osteoclast activity between patients and controls could be influenced by the menopausal state or the use of bone active agents, although we expect that the number of patients using bone active agents was probably low, since osteoporosis is often undertreated, particularly during the time of the study (1984-1999). It was not possible to correct for these potential confounding factors, because the controls were anonymous donors and only gender, age and the time

of blood donation are known. Additional information on the preclinical RA patients was collected by chart review and also in this group, menopausal state or the use of bone active agents could not be measured reliably. Since the controls were matched for age and gender, it is not expected that this has influenced the difference between patients and controls, although it could have influenced the absolute test values in both groups. Also, it was not possible to use fasting samples for this study since the blood bank recommends donors to eat before they donate blood. It is unknown whether this has influenced the results. Furthermore, data from only 71 patients could be used to study the association between preclinical levels of serum bone markers and regulators of osteoclast activity and radiographic progression after the start of the symptoms of RA. This could be the reason for the non-significant associations between the preclinical bone markers and radiographic progression in this study. Finally, it must be noted that the findings of this study can be used only in a population of patients and are not suitable for decision-making in individual patient care.

In conclusion, the present study of the asymptomatic phase of RA adds evidence of an alteration of bone metabolism parallel to the increased inflammation and autoimmunity described earlier in this situation. In at least a part of the patients, the process of joint destruction is already underway when they experience the first symptoms of the disease.

References

1. Jansen LM, van Schaardenburg D, van der Horst-Bruinsma IE, Bezemer PD, Dijkmans BA. Predictors of functional status in patients with early rheumatoid arthritis. Ann Rheum Dis 2000; 59(3):223-226.
2. van der Heijde DM. Joint erosions and patients with early rheumatoid arthritis. Br J Rheumatol 1995; 34 Suppl 2:74-78.
3. Kuper HH, van Leeuwen MA, van Riel PL, Prevoo ML, Houtman PM, Lolkema WF et al. Radiographic damage in large joints in early rheumatoid arthritis: relationship with radiographic damage in hands and feet, disease activity, and physical disability. Br J Rheumatol 1997; 36(8):855-860.
4. Drossaers-Bakker KW, Kroon HM, Zwinderman AH, Breedveld FC, Hazes JM. Radiographic damage of large joints in long-term rheumatoid arthritis and its relation to function. Rheumatology (Oxford) 2000; 39(9):998-1003.
5. Boers M, Verhoeven AC, Markusse HM, van de Laar MA, Westhovens R, van Denderen JC et al. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 1997; 350(9074):309-318.
6. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, van ZD, Kerstens PJ, Hazes JM et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): a randomized, controlled trial. Arthritis Rheum 2005; 52(11):3381-3390.
7. Garnero P, Jouvenne P, Buchs N, Delmas PD, Miossec P. Uncoupling of bone metabolism in rheumatoid arthritis patients with or without joint destruction: assessment with serum type I collagen breakdown products. Bone 1999; 24(4):381-385.
8. Jansen LM, van der Horst-Bruinsma I, Lems WF, van Schaardenburg D, van de Stad R, de Koning M et al. Serological bone markers and joint damage in early polyarthritis. J Rheumatol 2004; 31(8):1491-1496.
9. Cortet B, Flipo RM, Pigny P, Duquesnoy B, Boersma A, Marchandise X et al. Is bone turnover a determinant of bone mass in rheumatoid arthritis? J Rheumatol 1998; 25(12):2339-2344.
10. Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 2002; 46(1):21-30.
11. Garnero P, Landewe R, Boers M, Verhoeven A, van der Linden S, Christgau S et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. Arthritis Rheum 2002; 46(11):2847-2856.
12. Landewe R, Geusens P, Boers M, van der Heijde D, Lems W, te Koppele J et al. Markers for type II collagen breakdown predict the effect of disease-modifying treatment on long-term radiographic progression in patients with rheumatoid arthritis. Arthritis Rheum 2004; 50(5):1390-1399.

13. Ziolkowska M, Kurowska M, Radzikowska A, Luszczykiewicz G, Wiland P, Dziewczopolski W et al. High levels of osteoprotegerin and soluble receptor activator of nuclear factor kappa B ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor alpha treatment. Arthritis Rheum 2002; 46(7):1744-1753.

14. Geusens PP, Landewe RB, Garnero P, Chen D, Dunstan CR, Lems WF et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. Arthritis Rheum 2006; 54(6):1772-1777.

15. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004; 50(2):380-386.

16. Nielen MM, van Schaardenburg D, Reesink HW, Twisk JW, van de Stadt RJ, van der Horst-Bruinsma IE et al. Increased levels of C-reactive protein in serum from blood donors before the onset of rheumatoid arthritis. Arthritis Rheum 2004; 50(8):2423-2427.

17. Nielen MM, van Schaardenburg D, Reesink HW, Twisk JW, van de Stadt RJ, van der Horst-Bruinsma IE et al. Simultaneous development of acute phase response and autoantibodies in preclinical rheumatoid arthritis. Ann Rheum Dis 2006; 65(4):535-537.

18. Twisk JW. Applied longitudinal data analysis for epidemiology: a practical guide. Cambridge (UK): Cambridge University Press; 2003.

19 Molenaar ET, Lems WF, Dijkmans BA, de Koning MH, van de Stadt RJ, Voskuyl AE. Levels of markers of bone resorption are moderately increased in patients with inactive rheumatoid arthritis. Rheumatology (Oxford) 2000; 39(7):742-744.

20. Hayer S, Redlich K, Korb A, Hermann S, Smolen J, Schett G. Tenosynovitis and osteoclast formation as the initial preclinical changes in a murine model of inflammatory arthritis. Arthritis Rheum 2006; 56(1):79-88