

BMJ Open

Rheumatoid factor and anti-CCP do not predict progressive joint damage in patients with early rheumatoid arthritis treated with prednisolone – a randomized study

Journal:	BMJ Open
Manuscript ID:	bmjopen-2014-005246
Article Type:	Research
Date Submitted by the Author:	12-Mar-2014
Complete List of Authors:	Hafström, Ingiöld; Karolinska Institutet, Rheumatology dept Engvall, Inga-Lill; Karolinska Institutet, Rheumatology dept Rönnelid, Johan; Genetics and Pathology, Uppsala University, Department of Immunology Boonen, Annelies; Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, Department of Internal Medicine van der Heijde, Désirée; Leiden University Medical Center, Leiden, Department of Rheumatology Svensson, Björn; University of Lund, Sweden, Rheumatology
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Qualitative research
Keywords:	RHEUMATOLOGY, Clinical trials < THERAPEUTICS, Diagnostic radiology < RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

**Rheumatoid factor and anti-CCP do not predict
progressive joint damage in patients with early
rheumatoid arthritis treated with prednisolone – a
randomized study**

Ingiäld Hafström, Inga-Lill Engvall, Johan Rönnelid, Annelies Boonen, Désirée van der Heijde, Björn Svensson on behalf of the BARFOT study group

Ingiäld Hafström, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Inga-Lill Engvall, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Johan Rönnelid, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden;

Annelies Boonen, Department of Internal Medicine, Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, The Netherlands;

Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;

Björn Svensson, Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund, Sweden.

Corresponding author: Ingiäld Hafström, Rheumatology department, R92, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. Telephone +46 8 58581831, fax +46 8 58587260; e-mail ingiald.hafstrom@karolinska.se

Keywords: Rheumatoid arthritis, RF, anti-CCP, radiographic progression, prednisolone

Word count: 1504

Abstract

Objective. To analyse if predictors of radiographic progression differ between patients treated with or without prednisolone in early rheumatoid arthritis (RA). Radiographs of hands and feet were assessed using the modified Sharp/van der Heijde score and radiographic progression was defined as an increase in total Sharp score above 5.8 (the smallest-detectable-change).

Design. Prospective, randomized study of patients with early RA.

Setting. Secondary level of care; six participating centres from southern Sweden; both urban and rural populations.

Participants. In all 225 patients, 64% women, with a diagnosis of RA according to the American College of Rheumatology criteria were included if they were between 18 and 80 years of age and had a disease duration of less than one year.

Intervention. The patients were randomised to 7.5 mg prednisolone daily for two years (P-group; n=108) or no prednisolone, (NoP-group; n=117) when they started with their first DMARD and were prospectively followed for two years.

Results. The frequency of patients with radiographic progression after two years was 26% in the P-group and 39% in the NoP-group ($p=0.033$). Relevant interactions between treatment and RF ($p=0.061$) and between treatment and anti-CCP ($p=0.096$) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), $p=0.001$, and OR (95%CI) 8.7 (2.5-31.3), $p=0.001$, respectively.

Conclusion. Presence of RF and anti-CCP predicted radiographic progression in patients not treated with prednisolone but failed to predict progression in patients treated with this drug. The data suggest that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms.

Trial registration: ISRCTN20612367

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Strengths and limitation of this study

- A strength of the study is the prospective design with randomization of patients with early RA to treatment with low-dose prednisolone or no prednisolone together with disease modifying anti-rheumatic drug for two years.
- Another strength is that most patients followed the treatment they were randomized to.
- The main limitation is the rather small number of patients in each subgroup, which may reduce statistical power.

• Introduction

Recent treatment strategies in early rheumatoid arthritis (RA) have considerably improved outcome. Nevertheless, most clinical trials as well as clinical practice show significant subgroups of patients who fail to respond and develop progressive joint damage.

In the BARFOT (Better Anti-Rheumatic Pharmacotherapy) low dose prednisolone study on 250 early (< 1 year disease duration) RA patients, joint damage progression was less frequent after two years in the group of patients who in addition to disease-modifying anti-rheumatic drugs (DMARDs) got prednisolone 7.5 mg daily compared to those treated with DMARDs alone.[1] Despite this achievement some patients in the prednisolone group deteriorated radiographically while some in the non-prednisolone group did not.

We therefore wanted to study if predictors of radiographic progression differed between patients treated with or without prednisolone in early RA.

Methods

Patients

The patients had all participated in the BARFOT low-dose prednisolone study in which radiographic progression was the primary outcome.[1] DMARDs were chosen by the treating physicians with the goal to achieve remission, defined as a Disease Activity Score (DAS28) <2.6. In addition, the patients were randomized to prednisolone, 7.5 mg/day, (P-group n=119) or no prednisolone (NoP-group n=131).

The present study population consisted of the 225 (90% of the randomized) patients who had radiographs of hands and feet at both baseline and the 2-year follow-up. Of these, 108 patients were in the P-group and 117 in the NoP-group.

All patients gave their informed consent and the ethics committees approved the study, which was performed in accordance with the Helsinki Declaration.

Radiographic assessment

Radiographs were scored for erosions, joint space narrowing and total Sharp scores (TSS) with known time sequence using the van der Heijde modification of the Sharp score, by two readers.[2] The smallest detectable change (SDC), based on interobserver data, was calculated to be 5.8 [1] admitting radiographic progressors to be defined as having a TSS >5.8.

Disease activity and physical function

Disease activity was assessed by DAS28.[3] The Swedish version of the Stanford Health Assessment Questionnaire (HAQ) was used to measure daily life function.[4]

Laboratory analyses

Plasma and serum samples were stored at -70°C until assay. IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide 2 (anti-CCP) were analysed using enzyme immunoassay (Phadia 250, Thermofisher AB, Uppsala, Sweden). Levels of ≥ 5 international units (IU)/ml (IgM RF) and ≥ 7 arbitrary units (AU)/ml (anti-CCP) were regarded as positive. Samples from individual patients were analysed in parallel. When 100 healthy blood donor controls were analyzed in the same laboratory, 4 were IgM RF positive and none were anti-CCP positive, corresponding to 96% and 100% specificity, respectively.

Procollagen type I N-terminal propeptide (PINP; marker of bone formation), C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (ICTP; both markers of bone degradation) were analysed as described earlier.[5]

Statistics

The SPSS V.21.0 statistical software was used. To test differences between groups, the Mann–Whitney U test, or unpaired t test was used for continuous variables, whereas the Wilcoxon matched pairs test was used for paired comparisons and the χ^2 test for proportions. Two-tailed p values <0.05 were regarded as significant. To identify predictors of radiographic progression, baseline clinical and demographic variables with $p<0.10$ in univariate analyses were entered into multiple logistic regression models. Prediction analyses in subgroups were justified by interaction analyses (relevant interaction $p<0.1$).

Results

Radiographic progression

After 2 years the frequency of patients with radiographic progression (progressors) was 26% in the P-group and 39% in the NoP-group, $p=0.033$.

Baseline characteristics and associations between baseline variables and radiographic progression

Demographic and clinical characteristics at baseline in patients with and without progression of joint damage after 2 years are shown in table 1. Univariate analyses per treatment group showed that in the P-group progressors had significantly more swollen joints and higher TSS

than non-progressors, whereas in the NoP-group presence of RF and anti-CCP as well as elevated CRP and TSS were associated with radiographic progression. The concentrations of PINP, CTX-1 and ICTP did not differ significantly between progressors and non-progressors, irrespective of prednisolone treatment.

Table 1. Demographic and clinical characteristics at baseline separated into patients randomized to prednisolone (P-group, n=108) and no prednisolone (NoP-group, n=117) and further separated into those who after 2 years had progression in total Sharp score >5.8 or not, progressors and non- progressors, respectively.

Baseline characteristics	P-group			NoP-group		
	Progressors n=28	Non-progressors n=80	p-value	Progressors n=46	Non-progressors n=71	p-value
Age, years	50 (13)	52 (15)	0.57	58 (13)	58 (13)	0.89
Women, n (%)	17 (61)	52 (65)	0.68	29 (63)	46 (65)	0.85
Smokers						
ever, %	78.6	61.3	0.10	63.0	60.0	0.74
never, %	21.4	38.8		37.0	40.0	
Disease dur. mo	7 (3)	6 (4)	0.83	6 (3)	6 (3)	0.22
RF pos, n (%)	20 (74.1)	38 (54.3)	0.075	33 (86.8)	30 (48.4)	0.001
Anti-CCP pos, n (%)	20 (74.1)	41 (58.6)	0.157	31 (81.6)	28 (45.2)	0.001
DAS28	5.33 (1.34)	5.23 (1.02)	0.69	5.44 (1.06)	5.45 (0.98)	0.94
ESR, mm	41 (24)	36 (26)	0.38	43 (23)	34 (25)	0.06
Swollen joints, n	13 (5)	11 (5)	0.029	11 (6)	11 (5)	0.82
Tender joints, n	8 (7)	7 (5)	0.88	8 (7)	9 (6)	0.26
General health, VAS, mm	39 (29)	47 (21)	0.14	43 (23)	48 (24)	0.23
CRP (mg/L)	38 (31)	30 (30)	0.08 [†]	43 (38)	31 (37)	0.012[†]
Pain, VAS, mm	44 (25)	48 (22)	0.38	47 (20)	50 (22)	0.39
HAQ (0-3)	0.94 (0.71)	1.01 (0.53)	0.56	1.13 (0.58)	0.9 (69)	0.07
TSS	5.37 (6.11)	3.67 (10.16)	0.033[†]	8.50 (13.13)	2.53 (5.53)	0.001[†]
PINP	33 (16)	22 (9)	0.074	48 (12)	49 (21)	0.82
CTX-1	0.26 (0.14)	5.1 (0.15)	0.15	0.35 (0.18)	0.33 (0.19)	0.82
ICTP	4.1 (1.8)	5.9 (7.7)	0.54	5.1 (1.4)	5.2 (2.6)	0.93

p- values represent differences between progressors and no progressors. Values are mean (SD). n = numbers; mo = months; Swollen and tender joints were calculated on 28 joints. VAS = visual analogue scale; TSS = Total Sharp score. P1NP = procollagen type I N-terminal propeptide, CTX-1 = C-terminal telopeptide crosslaps, 1CTP = C-terminal telopeptides of type I collagen. [†]Mann-Whitney U test.

Prednisolone and concomitant treatment

In the P-group, some patients reduced the prednisolone dose and 8 stopped treatment. In the NoP-group, 6 patients started prednisolone treatment during the study period. DMARD treatment (mostly methotrexate and sulphasalazine) was given to all patients and did not differ between progressors and non-progressors neither in the P-group nor in the NoP-group during the first three months.

Prediction of radiographic progression.

In addition to RF and anti-CCP, baseline swollen joint count, ESR, CRP, HAQ and TSS were univariately associated with radiographic progression ($p<0.1$) and were entered into multivariate logistic models.

Relevant interactions between treatment and RF ($p=0.061$) and between treatment and anti-CCP ($p=0.096$) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), $p=0.001$, and OR (95%CI) 8.7 (2.5-31.3), $p=0.001$, respectively.

Change in RF and anti-CCP during two years follow-up

In both treatment groups most patients retained their RF and anti-CCP status (pos/neg) during the two study years; for the P-group, 82.3% and 87.5%, respectively, and for the NoP-group, 88.9% and 98%, respectively. Some patients, however, reversed from RF and/or anti-CCP positivity to negativity; in the P-group 15.6% and 9.4%, respectively, and in the No-P-group 9.9% and 2.0%, respectively. More patients lost than acquired seropositivity.

RF and anti-CCP levels among seropositive patients did not differ between the treatment groups at baseline or at 2 years, but in both treatment groups there were significant reductions in both autoantibody levels during the study period (table 2). When calculated only on those patients who were compliant with the randomization and dose of

prednisolone, the P-group had a larger reduction of anti-CCP than the NoP-group, $p=0.028$ (table 2).

Table 2. Levels of RF and anti-CCP (median (IQR)) in the patients positive for one or both of these antibodies in the two treatment groups.

	P-group (n=97)	NoP-group (n=100)	P-value between groups	P-value between groups, only patients with dose according to protocol (77 vs 94)
RF, baseline (IU/ml)	12.0 (1.3-58.0)	21.5 (1.9-80.5)	0.39	0.91
Anti-CCP, baseline (AU/ml)	28.0 (3.4-367.0)	43.5 (2.5-384.5)	0.63	0.38
RF, 2 years	4.1 (0.7-28.0)	9.5 (1.00-52.0)	0.14	0.41
Anti-CCP, 2 years	13.0 (2.3-141.0)	24.0(2.1-446.0)	0.70	1.00
Δ RF, 0-2 years	-1.1 (-20.3-0.20)**	-1.5 (-34.0-0)**	0.63	0.69
Δ Anti-CCP, 0-2 years	-1.9 (-55.4- -0.10) **	-0.3 (-88.0- 0.5)*	0.14	0.028

*= $p<0.05$, **= $p<0.001$ (Wilcoxon matched pairs test).

Discussion

The present study was undertaken to analyse if predictors for radiographic progression differed between early RA patients treated with or without prednisolone, in combination with DMARDs during the first two years after diagnosis. The main finding was that RF and anti-CCP predicted radiographic progression only in the group not treated with prednisolone.

The presence of RF and antibodies against citrullinated proteins/peptides (ACPA) has been found to predict the development of RA and also the severity of the disease, suggesting a possible pathogenic role for these autoantibodies.[6-8] If so, the present finding that RF- and anti-CCP-positivity did not predict radiographic progression in prednisolone treated patients may imply that prednisolone affects the pathogenic mechanisms associated with these antibodies in early RA. This possibility is in line with a role for RF in joint damage progression beyond its direct effect on disease activity.[9] Interestingly, such effects of RF, independent of disease activity, have been shown to be significantly associated only with progression of the erosion score, but not with the joint space narrowing score.[9] Similarly, we have earlier reported that the hampering effect of prednisolone on radiographic progression was valid only for erosions.[10]

The lack of association between autoantibody status and radiographic progression in the prednisolone treated patients is consistent with similar findings in patients treated with some biological agents.[11-12] It is further in line with the findings in the BEST study where the association of ACPA-status with joint damage progression was significantly more pronounced in patients treated with initial methotrexate monotherapy compared with those getting combination therapy with prednisolone or anti-TNF agents.[13] One explanation might be that early and intensive reduction of inflammation, also found here in the P-group, may suppress a strong autoimmune response.[14]

Such an explanation to the fact that the autoantibodies at baseline did not predict radiographic progression in the P-group is supported by the finding that more patients in this group reverted from seropositivity to negativity. In a recent study by Barra et al on early inflammatory arthritis, seroreversion occurred in rates similar to those in the present study without any influence on the prediction of outcome.[15] However, in another early polyarthritis cohort the prognostic significance of initial RF and anti-CCP positivity was influenced by seroreversion of these antibodies.[16]

Not only antibody status but also serum level changes might be of importance in the prediction of outcome. Here we found that the levels of RF and anti-CCP decreased in both treatment groups. This decrease was significantly more profound in the P-group only if the calculation was based on the patients who strictly followed randomisation and dose. We suggest that such a subgroup analysis is important to find specific effects of prednisolone. Reports on the predictive value of changes in autoantibody levels are limited, but one study

on early RA reports that changes in RF and ACPA levels were not associated with radiographic outcome.[17] In established RA, RF and ACPA level reductions are reported to be closely linked to treatment-associated improvements.[18] However, if such reductions are associated with structural changes remains unknown.

In conclusion, presence of RF and anti-CCP did not predict radiographic progression in patients treated with prednisolone in contrast to prednisolone-naïve patients. The data imply that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms. The clinical implication would be that the unfavourable prognosis associated with RF- and anti-CCP- positivity can be relieved by prednisolone treatment.

Acknowledgements

We thank research nurse Margareta Wörnert for excellent assistance, MD Kristina Albertsson for scoring radiographs and the following members of the BARFOT study group: Maria Andersson, Kristina Forslind, Catharina Keller, Ido Leden, Bengt Lindell, Ingemar Petersson, Christoffer Schaufelberger, Annika Teleman and Jan Theander.

Competing interest

None

Contributor statement

IH, AB, DvH and BS were involved in the original planning of the low-dose prednisolone study and interpretation of data. IH was responsible for data acquisition and preparing the manuscript, AB for scoring the radiographs and BS and DvH for statistical analyses. ILE was involved in the statistical analyses and JR performed the analyses of RF and anti-CCP. All authors read and approved the manuscript.

Funding

This work was supported by grants from The Swedish Rheumatism Association, King Gustav V 80 year's Foundation, The Swedish Research Council, and through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet.

Data sharing statement

There is no additional data available.

For peer review only

References

1. Svensson B, Boonen A, Albertsson K, et al. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;**52**:3360-70.
2. van der Heijde DM, van Leeuwen MA, van Riel PL, et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;**35**:26-34.
3. Prevoo ML, van 't Hof MA, Kuper HH, et al. 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. Ekdahl C, Eberhardt K, Andersson SI, et al. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scand J Rheumatol* 1988;**17**:263-71.
5. Engvall IL, Svensson B, Tengstrand B, et al. Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. *Arthritis Res Ther* 2008;**10**:R128.
6. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2741-9.
7. van der Heijde DM, van Riel PL, van Leeuwen MA, et al. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. *British J Rheumatol* 1992;**31**:519-25.
8. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;**7**:R949-58.
9. Aletaha D, Alasti F, Smolen JS. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann Rheum Dis* 2013;**72**:875-80.
10. Engvall IL, Svensson B, Boonen A, et al. Low-dose prednisolone in early rheumatoid arthritis inhibits collagen type I degradation by matrix metalloproteinases as assessed by

serum ICTP--a possible mechanism for specific inhibition of radiological destruction. *Rheumatol* 2013;**52**:733-42.

11. Burmester GR, Feist E, Kellner H, et al. Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA). *Ann Rheum Dis* 2011;**70**:755-59.

12. Soto L, Sabugo F, Catalan D, et al. The presence of anti-citrullinated protein antibodies (ACPA) does not affect the clinical response to adalimumab in a group of RA patients with the tumor necrosis factor (TNF) alpha-308 G/G promoter polymorphism. *Clin Rheumatol* 2011;**30**:391-5.

13. van den Broek M, Dirven L, Klarenbeek NB, et al. The association of treatment response and joint damage with ACPA-status in recent-onset RA: a subanalysis of the 8-year follow-up of the BeSt study. *Ann Rheum Dis* 2012;**71**:245-8.

14. de Vries-Bouwstra JK, Goekoop-Ruiterman YPM, Verpoort KN, et al. Progression of joint damage in early rheumatoid arthritis. Association with HLA-DRB1, rheumatoid factor, and anti-citrullinated protein antibodies in relation to different treatment strategies. *Arthritis Rheum* 2008;**58**:1293-8.

15. Barra L, Bykerk V, Pope JE, et al. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;**40**:1259-67.

16. Guzman MC, Carrier N, Cossette P, et al. Outcomes in recent-onset inflammatory polyarthritis differ according to initial titers, persistence over time, and specificity of the autoantibodies. *Arthritis Care Res* 2010;**62**:1624-32.

17. Ursum J, Bos WH, van Dillen N, et al. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010;**12**:R8.

18. Bohler C, Radner H, Smolen JS, et al. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 2013;**72**:241-4.

Rheumatoid factor and anti-CCP do not predict progressive joint damage in patients with early rheumatoid arthritis treated with prednisolone – a randomized study

Ingjald Hafström, Inga-Lill Engvall, Johan Rönnelid, Annelies Boonen, Désirée van der Heijde, Björn Svensson on behalf of the BARFOT study group

Ingjald Hafström, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Inga-Lill Engvall, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Johan Rönnelid, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden;

Annelies Boonen, Department of Internal Medicine, Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, The Netherlands;

Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;

Björn Svensson, Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund, Sweden.

Corresponding author: Ingjald Hafström, Rheumatology department, R92, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. Telephone +46 8 58581831, fax +46 8 58587260; e-mail ingjald.hafstrom@karolinska.se

Keywords: Rheumatoid arthritis, RF, anti-CCP, radiographic progression, prednisolone

Word count: 1504

Abstract

Objective. To analyse if predictors of radiographic progression differ between patients treated with or without prednisolone in early rheumatoid arthritis (RA). Radiographs of hands and feet were assessed using the modified Sharp/van der Heijde score and radiographic progression was defined as an increase in total Sharp score above 5.8 (the smallest-detectable-change).

Design. Prospective, randomized study of patients with early RA.

Setting. Secondary level of care; six participating centres from southern Sweden; both urban and rural populations.

Participants. In all 225 patients, 64% women, with a diagnosis of RA according to the American College of Rheumatology criteria were included if they were between 18 and 80 years of age and had a disease duration of less than one year.

Intervention. The patients were randomised to 7.5 mg prednisolone daily for two years (P-group; n=108) or no prednisolone, (NoP-group; n=117) when they started with their first DMARD and were prospectively followed for two years.

Results. The frequency of patients with radiographic progression after two years was 26% in the P-group and 39% in the NoP-group (p=0.033). Relevant interactions between treatment and RF (p=0.061) and between treatment and anti-CCP (p=0.096) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), p=0.001, and OR (95%CI) 8.7 (2.5-31.3), p=0.001, respectively.

Conclusion. Presence of RF and anti-CCP predicted radiographic progression in patients not treated with prednisolone but failed to predict progression in patients treated with this drug. The data suggest that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms.

Trial registration: ISRCTN20612367

Strengths and limitation of this study

- A strength of the study is the prospective design with randomization of patients with early RA to treatment with low-dose prednisolone or no prednisolone together with disease modifying anti-rheumatic drug for two years.
- Another strength is that most patients followed the treatment they were randomized to.
- The main limitation is the rather small number of patients in each subgroup, which may reduce statistical power.

1
2
3
4
5
6
7
8
9
• Introduction

Recent treatment strategies in early rheumatoid arthritis (RA) have considerably improved outcome. Nevertheless, most clinical trials as well as clinical practice show significant subgroups of patients who fail to respond and develop progressive joint damage.

10
11
12
13
14
15
16
17
18
19
In the BARFOT (Better Anti-Rheumatic Pharmacotherapy) low dose prednisolone study on 250 early (< 1 year disease duration) RA patients, joint damage progression was less frequent after two years in the group of patients who in addition to disease-modifying anti-rheumatic drugs (DMARDs) got prednisolone 7.5 mg daily compared to those treated with DMARDs alone.[1] Despite this achievement some patients in the prednisolone group deteriorated radiographically while some in the non-prednisolone group did not.

20
21
22
23
24
We therefore wanted to study if predictors of radiographic progression differed between patients treated with or without prednisolone in early RA.

25
26
27
Methods

28
Patients

29
30
31
32
33
34
35
36
The patients had all participated in the BARFOT low-dose prednisolone study in which radiographic progression was the primary outcome.[1] DMARDs were chosen by the treating physicians with the goal to achieve remission, defined as a Disease Activity Score (DAS28) <2.6. In addition, the patients were randomized to prednisolone, 7.5 mg/day, (P-group n=119) or no prednisolone (NoP-group n=131).

37
38
39
40
41
42
The present study population consisted of the 225 (90% of the randomized) patients who had radiographs of hands and feet at both baseline and the 2-year follow-up. Of these, 108 patients were in the P-group and 117 in the NoP-group.

43
44
45
46
All patients gave their informed consent and the ethics committees approved the study, which was performed in accordance with the Helsinki Declaration.

47
Radiographic assessment

48
49
50
51
52
53
54
55
56
57
58
59
60
Radiographs were scored for erosions, joint space narrowing and total Sharp scores (TSS) with known time sequence using the van der Heijde modification of the Sharp score, by two readers.[2] The smallest detectable change (SDC), based on interobserver data, was calculated to be 5.8 [1] admitting radiographic progressors to be defined as having a TSS >5.8.

Disease activity and physical function

Disease activity was assessed by DAS28.[3] The Swedish version of the Stanford Health Assessment Questionnaire (HAQ) was used to measure daily life function.[4]

Laboratory analyses

Plasma and serum samples were stored at -70°C until assay. IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide 2 (anti-CCP) were analysed using enzyme immunoassay (Phadia 250, Thermofisher AB, Uppsala, Sweden). Levels of ≥ 5 international units (IU)/ml (IgM RF) and ≥ 7 arbitrary units (AU)/ml (anti-CCP) were regarded as positive. Samples from individual patients were analysed in parallel. When 100 healthy blood donor controls were analyzed in the same laboratory, 4 were IgM RF positive and none were anti-CCP positive, corresponding to 96% and 100% specificity, respectively.

Procollagen type I N-terminal propeptide (P1NP; marker of bone formation), C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (ICTP; both markers of bone degradation) were analysed as described earlier.[5]

Statistics

The SPSS V.21.0 statistical software was used. To test differences between groups, the Mann–Whitney U test, or unpaired t test was used for continuous variables, whereas the Wilcoxon matched pairs test was used for paired comparisons and the χ^2 test for proportions. Two-tailed p values < 0.05 were regarded as significant. To identify predictors of radiographic progression, baseline clinical and demographic variables with $p < 0.10$ in univariate analyses were entered into multiple logistic regression models. Prediction analyses in subgroups were justified by interaction analyses (relevant interaction $p < 0.1$).

Results

Radiographic progression

After 2 years the frequency of patients with radiographic progression (progressors) was 26% in the P-group and 39% in the NoP-group, $p = 0.033$.

Baseline characteristics and associations between baseline variables and radiographic progression

Demographic and clinical characteristics at baseline in patients with and without progression of joint damage after 2 years are shown in table 1. Univariate analyses per treatment group showed that in the P-group progressors had significantly more swollen joints and higher TSS

than non-progressors, whereas in the NoP-group presence of RF and anti-CCP as well as elevated CRP and TSS were associated with radiographic progression. The concentrations of P1NP, CTX-1 and ICTP did not differ significantly between progressors and non-progressors, irrespective of prednisolone treatment.

Table 1. Demographic and clinical characteristics at baseline separated into patients randomized to prednisolone (P-group, n=108) and no prednisolone (NoP-group, n=117) and further separated into those who after 2 years had progression in total Sharp score >5.8 or not, progressors and non- progressors, respectively.

Baseline characteristics	P-group			NoP-group		
	Progressors n=28	Non-progressors n=80	p-value	Progressors n=46	Non-progressors n=71	p-value
Age, years	50 (13)	52 (15)	0.57	58 (13)	58 (13)	0.89
Women, n (%)	17 (61)	52 (65)	0.68	29 (63)	46 (65)	0.85
Smokers						
ever, %	78.6	61.3	0.10	63.0	60.0	0.74
never, %	21.4	38.8		37.0	40.0	
Disease dur. mo	7 (3)	6 (4)	0.83	6 (3)	6 (3)	0.22
RF pos, n (%)	20 (74.1)	38 (54.3)	0.075	33 (86.8)	30 (48.4)	0.001
Anti-CCP pos, n (%)	20 (74.1)	41 (58.6)	0.157	31 (81.6)	28 (45.2)	0.001
DAS28	5.33 (1.34)	5.23 (1.02)	0.69	5.44 (1.06)	5.45 (0.98)	0.94
ESR, mm	41 (24)	36 (26)	0.38	43 (23)	34 (25)	0.06
Swollen joints, n	13 (5)	11 (5)	0.029	11 (6)	11 (5)	0.82
Tender joints, n	8 (7)	7 (5)	0.88	8 (7)	9 (6)	0.26
General health, VAS, mm	39 (29)	47 (21)	0.14	43 (23)	48 (24)	0.23
CRP (mg/L)	38 (31)	30 (30)	0.08 [†]	43 (38)	31 (37)	0.012 [†]
Pain, VAS, mm	44 (25)	48 (22)	0.38	47 (20)	50 (22)	0.39
HAQ (0-3)	0.94 (0.71)	1.01 (0.53)	0.56	1.13 (0.58)	0.9 (69)	0.07
TSS	5.37 (6.11)	3.67 (10.16)	0.033 [†]	8.50 (13.13)	2.53 (5.53)	0.001 [†]
P1NP	33 (16)	22 (9)	0.074	48 (12)	49 (21)	0.82
CTX-1	0.26 (0.14)	5.1 (0.15)	0.15	0.35 (0.18)	0.33 (0.19)	0.82
ICTP	4.1 (1.8)	5.9 (7.7)	0.54	5.1 (1.4)	5.2 (2.6)	0.93

p- values represent differences between progressors and no progressors. Values are mean (SD). n = numbers; mo = months; Swollen and tender joints were calculated on 28 joints. VAS = visual analogue scale; TSS = Total Sharp score. PINP = procollagen type I N-terminal propeptide, CTX-1 = C-terminal telopeptide crosslaps, ICTP = C-terminal telopeptides of type I collagen. [†]Mann-Whitney U test.

Prednisolone and concomitant treatment

In the P-group, some patients reduced the prednisolone dose and 8 stopped treatment. In the NoP-group, 6 patients started prednisolone treatment during the study period. DMARD treatment (mostly methotrexate and sulphasalazine) was given to all patients and did not differ between progressors and non-progressors neither in the P-group nor in the NoP-group during the first three months.

Prediction of radiographic progression.

In addition to RF and anti-CCP, baseline swollen joint count, ESR, CRP, HAQ and TSS were univariately associated with radiographic progression ($p < 0.1$) and were entered into multivariate logistic models.

Relevant interactions between treatment and RF ($p = 0.061$) and between treatment and anti-CCP ($p = 0.096$) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), $p = 0.001$, and OR (95%CI) 8.7 (2.5-31.3), $p = 0.001$, respectively.

Change in RF and anti-CCP during two years follow-up

In both treatment groups most patients retained their RF and anti-CCP status (pos/neg) during the two study years; for the P-group, 82.3% and 87.5%, respectively, and for the NoP-group, 88.9% and 98%, respectively. Some patients, however, reversed from RF and/or anti-CCP positivity to negativity; in the P-group 15.6% and 9.4%, respectively, and in the No-P-group 9.9% and 2.0%, respectively. More patients lost than acquired seropositivity.

RF and anti-CCP levels among seropositive patients did not differ between the treatment groups at baseline or at 2 years, but in both treatment groups there were significant reductions in both autoantibody levels during the study period (table 2). When calculated only on those patients who were compliant with the randomization and dose of

prednisolone, the P-group had a larger reduction of anti-CCP than the NoP-group, p=0.028 (table 2).

Table 2. Levels of RF and anti-CCP (median (IQR)) in the patients positive for one or both of these antibodies in the two treatment groups.

	P-group (n=97)	NoP-group (n=100)	P-value between groups	P-value between groups, only patients with dose according to protocol (77 vs 94)
RF, baseline (IU/ml)	12.0 (1.3-58.0)	21.5 (1.9-80.5)	0.39	0.91
Anti-CCP, baseline (AU/ml)	28.0 (3.4-367.0)	43.5 (2.5-384.5)	0.63	0.38
RF, 2 years	4.1 (0.7-28.0)	9.5 (1.00-52.0)	0.14	0.41
Anti-CCP, 2 years	13.0 (2.3-141.0)	24.0(2.1-446.0)	0.70	1.00
Δ RF, 0-2 years	-1.1 (-20.3-0.20)**	-1.5 (-34.0-0)**	0.63	0.69
Δ Anti-CCP, 0-2 years	-1.9 (-55.4- -0.10) **	-0.3 (-88.0- 0.5)*	0.14	0.028

*= p<0.05, **= p<0.001 (Wilcoxon matched pairs test).

Discussion

The present study was undertaken to analyse if predictors for radiographic progression differed between early RA patients treated with or without prednisolone, in combination with DMARDs during the first two years after diagnosis. The main finding was that RF and anti-CCP predicted radiographic progression only in the group not treated with prednisolone.

The presence of RF and antibodies against citrullinated proteins/peptides (ACPA) has been found to predict the development of RA and also the severity of the disease, suggesting a possible pathogenic role for these autoantibodies.[6-8] If so, the present finding that RF- and anti-CCP-positivity did not predict radiographic progression in prednisolone treated patients may imply that prednisolone affects the pathogenic mechanisms associated with these antibodies in early RA. This possibility is in line with a role for RF in joint damage progression beyond its direct effect on disease activity.[9] Interestingly, such effects of RF, independent of disease activity, have been shown to be significantly associated only with progression of the erosion score, but not with the joint space narrowing score.[9] Similarly, we have earlier reported that the hampering effect of prednisolone on radiographic progression was valid only for erosions.[10]

The lack of association between autoantibody status and radiographic progression in the prednisolone treated patients is consistent with similar findings in patients treated with some biological agents.[11-12] It is further in line with the findings in the BEST study where the association of ACPA-status with joint damage progression was significantly more pronounced in patients treated with initial methotrexate monotherapy compared with those getting combination therapy with prednisolone or anti-TNF agents.[13] One explanation might be that early and intensive reduction of inflammation, also found here in the P-group, may suppress a strong autoimmune response.[14]

Such an explanation to the fact that the autoantibodies at baseline did not predict radiographic progression in the P-group is supported by the finding that more patients in this group reverted from seropositivity to negativity. In a recent study by Barra et al on early inflammatory arthritis, seroreversion occurred in rates similar to those in the present study without any influence on the prediction of outcome.[15] However, in another early polyarthritis cohort the prognostic significance of initial RF and anti-CCP positivity was influenced by seroreversion of these antibodies.[16]

Not only antibody status but also serum level changes might be of importance in the prediction of outcome. Here we found that the levels of RF and anti-CCP decreased in both treatment groups. This decrease was significantly more profound in the P-group only if the calculation was based on the patients who strictly followed randomisation and dose. We suggest that such a subgroup analysis is important to find specific effects of prednisolone. Reports on the predictive value of changes in autoantibody levels are limited, but one study

on early RA reports that changes in RF and ACPA levels were not associated with radiographic outcome.[17] In established RA, RF and ACPA level reductions are reported to be closely linked to treatment-associated improvements.[18] However, if such reductions are associated with structural changes remains unknown.

In conclusion, presence of RF and anti-CCP did not predict radiographic progression in patients treated with prednisolone in contrast to prednisolone-naïve patients. The data imply that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms. The clinical implication would be that the unfavourable prognosis associated with RF- and anti-CCP- positivity can be relieved by prednisolone treatment.

Acknowledgements

We thank research nurse Margareta Wörnert for excellent assistance, MD Kristina Albertsson for scoring radiographs and the following members of the BARFOT study group: Maria Andersson, Kristina Forslind, Catharina Keller, Ido Leden, Bengt Lindell, Ingemar Petersson, Christoffer Schaufelberger, Annika Teleman and Jan Theander.

Competing interest

None

Contributor statement

IH, AB, DvH and BS were involved in the original planning of the low-dose prednisolone study and interpretation of data. IH was responsible for data acquisition and preparing the manuscript, AB for scoring the radiographs and BS and DvH for statistical analyses. ILE was involved in the statistical analyses and JR performed the analyses of RF and anti-CCP. All authors read and approved the manuscript.

Funding

This work was supported by grants from The Swedish Rheumatism Association, King Gustav V 80 year's Foundation, The Swedish Research Council, and through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet.

Data sharing statement

There is no additional data available.

For peer review only

References

1. Svensson B, Boonen A, Albertsson K, et al. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;**52**:3360-70.

2. van der Heijde DM, van Leeuwen MA, van Riel PL, et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;**35**:26-34.

3. Prevoo ML, van 't Hof MA, Kuper HH, et al. 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. Ekdahl C, Eberhardt K, Andersson SI, et al. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scand J Rheumatol* 1988;**17**:263-71.

5. Engvall IL, Svensson B, Tengstrand B, et al. Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. *Arthritis Res Ther* 2008;**10**:R128.

6. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2741-9.

7. van der Heijde DM, van Riel PL, van Leeuwen MA, et al. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. *British J Rheumatol* 1992;**31**:519-25.

8. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;**7**:R949-58.

9. Aletaha D, Alasti F, Smolen JS. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann Rheum Dis* 2013;**72**:875-80.

10. Engvall IL, Svensson B, Boonen A, et al. Low-dose prednisolone in early rheumatoid arthritis inhibits collagen type I degradation by matrix metalloproteinases as assessed by

serum ICTP--a possible mechanism for specific inhibition of radiological destruction.

Rheumatol 2013;**52**:733-42.

11. Burmester GR, Feist E, Kellner H, et al. Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA). *Ann Rheum Dis* 2011;**70**:755-59.

12. Soto L, Sabugo F, Catalan D, et al. The presence of anti-citrullinated protein antibodies (ACPA) does not affect the clinical response to adalimumab in a group of RA patients with the tumor necrosis factor (TNF) alpha-308 G/G promoter polymorphism. *Clin Rheumatol* 2011;**30**:391-5.

13. van den Broek M, Dirven L, Klarenbeek NB, et al. The association of treatment response and joint damage with ACPA-status in recent-onset RA: a subanalysis of the 8-year follow-up of the BeSt study. *Ann Rheum Dis* 2012;**71**:245-8.

14. de Vries-Bouwstra JK, Goekoop-Ruiterman YPM, Verpoort KN, et al. Progression of joint damage in early rheumatoid arthritis. Association with HLA-DRB1, rheumatoid factor, and anti-citrullinated protein antibodies in relation to different treatment strategies. *Arthritis Rheum* 2008;**58**:1293-8.

15. Barra L, Bykerk V, Pope JE, et al. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;**40**:1259-67.

16. Guzman MC, Carrier N, Cossette P, et al. Outcomes in recent-onset inflammatory polyarthritis differ according to initial titers, persistence over time, and specificity of the autoantibodies. *Arthritis Care Res* 2010;**62**:1624-32.

17. Ursum J, Bos WH, van Dillen N, et al. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010;**12**:R8.

18. Bohler C, Radner H, Smolen JS, et al. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 2013;**72**:241-4.



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4, ref 1
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/a
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4, ref 1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4, ref 1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	4
Sample size	7a	How sample size was determined	Power calculation
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Ref 1
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Ref 1
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Ref1

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Ref 1
	11b	If relevant, description of the similarity of interventions	na
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	page
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Ref 1
	14b	Why the trial ended or was stopped	Ref 1
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Ref 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Ref 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Ref 1
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	na
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	na
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Ref 1
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	2
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	9-10
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	9
Other information			
Registration	23	Registration number and name of trial registry	ISRCTN2061 2367
Protocol	24	Where the full trial protocol can be accessed, if available	na
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	10

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

For peer review only

BMJ Open

Rheumatoid factor and anti-CCP do not predict progressive joint damage in patients with early rheumatoid arthritis treated with prednisolone – a randomized study

Journal:	BMJ Open
Manuscript ID:	bmjopen-2014-005246.R1
Article Type:	Research
Date Submitted by the Author:	16-Jun-2014
Complete List of Authors:	Hafström, Ingiöld; Karolinska Institutet, Rheumatology dept Engvall, Inga-Lill; Karolinska Institutet, Rheumatology dept Rönnelid, Johan; Genetics and Pathology, Uppsala University, Department of Immunology Boonen, Annelies; Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, Department of Internal Medicine van der Heijde, Désirée; Leiden University Medical Center, Leiden, Department of Rheumatology Svensson, Björn; University of Lund, Sweden, Rheumatology
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Qualitative research
Keywords:	RHEUMATOLOGY, Clinical trials < THERAPEUTICS, Diagnostic radiology < RADIOLOGY & IMAGING

SCHOLARONE™
Manuscripts

Rheumatoid factor and anti-CCP do not predict progressive joint damage in patients with early rheumatoid arthritis treated with prednisolone – a randomized study

Ingjald Hafström, Inga-Lill Engvall, Johan Rönnelid, Annelies Boonen, Désirée van der Heijde, Björn Svensson on behalf of the BARFOT study group

Ingjald Hafström, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Inga-Lill Engvall, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Johan Rönnelid, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden;

Annelies Boonen, Department of Internal Medicine, Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, The Netherlands;

Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;

Björn Svensson, Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund, Sweden.

Corresponding author: Ingjald Hafström, Rheumatology department, R92, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. Telephone +46 8 58581831, fax +46 8 58587260; e-mail ingjald.hafstrom@karolinska.se

Keywords: Rheumatoid arthritis, RF, anti-CCP, radiographic progression, prednisolone

Word count: 1504

Abstract

Objective. To analyse if predictors of radiographic progression differ between patients treated with or without prednisolone in early rheumatoid arthritis (RA). Radiographs of hands and feet were assessed using the modified Sharp/van der Heijde score and radiographic progression was defined as an increase in total Sharp score above 5.8 (the smallest-detectable-change).

Design. Prospective, randomized study of patients with early RA.

Setting. Secondary level of care; six participating centres from southern Sweden; both urban and rural populations.

Participants. In all 225 patients, 64% women, with a diagnosis of RA according to the American College of Rheumatology criteria were included if they were between 18 and 80 years of age and had a disease duration of less than one year.

Intervention. The patients were randomised to 7.5 mg prednisolone daily for two years (P-group; n=108) or no prednisolone, (NoP-group; n=117) when they started with their first DMARD and were prospectively followed for two years.

Results. The frequency of patients with radiographic progression after two years was 26% in the P-group and 39% in the NoP-group ($p=0.033$). Relevant interactions between treatment and RF ($p=0.061$) and between treatment and anti-CCP ($p=0.096$) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), $p=0.001$, and OR (95%CI) 8.7 (2.5-31.3), $p=0.001$, respectively.

Conclusion. Presence of RF and anti-CCP predicted radiographic progression in patients not treated with prednisolone but failed to predict progression in patients treated with this drug. The data suggest that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms.

Trial registration: ISRCTN20612367

Strengths and limitation of this study

- A strength of the study is the prospective design with randomization of patients with early RA to treatment with low-dose prednisolone or no prednisolone together with disease modifying anti-rheumatic drug for two years.
- Another strength is that most patients followed the treatment they were randomized to.
- The main limitation is the rather small number of patients in each subgroup, which may reduce statistical power.

• Introduction

Recent treatment strategies in early rheumatoid arthritis (RA) have considerably improved outcome. Nevertheless, most clinical trials as well as clinical practice show significant subgroups of patients who fail to respond and develop progressive joint damage.

In the BARFOT (Better Anti-Rheumatic Pharmacotherapy) low dose prednisolone study on 250 early (< 1 year disease duration) RA patients, joint damage progression was less frequent after two years in the group of patients who in addition to disease-modifying anti-rheumatic drugs (DMARDs) got prednisolone 7.5 mg daily compared to those treated with DMARDs alone.[1] Despite this achievement some patients in the prednisolone group deteriorated radiographically while some in the non-prednisolone group did not.

We therefore wanted to study if predictors of radiographic progression differed between patients treated with or without prednisolone in early RA.

Methods

Patients

The patients had all participated in the BARFOT low-dose prednisolone study in which radiographic progression was the primary outcome.[1] DMARDs were chosen by the treating physicians with the goal to achieve remission, defined as a Disease Activity Score (DAS28) <2.6. In addition, the patients were randomized to prednisolone, 7.5 mg/day, (P-group n=119) or no prednisolone (NoP-group n=131).

The present study population consisted of the 225 (90% of the randomized) patients who had radiographs of hands and feet at both baseline and the 2-year follow-up. Of these, 108 patients were in the P-group and 117 in the NoP-group.

All patients gave their informed consent and the ethics committees approved the study, which was performed in accordance with the Helsinki Declaration.

Radiographic assessment

Radiographs were scored for erosions, joint space narrowing and total Sharp scores (TSS) with known time sequence using the van der Heijde modification of the Sharp score, by two readers.[2] The smallest detectable change (SDC), based on interobserver data, was calculated to be 5.8 [1] admitting radiographic progressors to be defined as having a TSS >5.8.

Disease activity and physical function

Disease activity was assessed by DAS28.[3] The Swedish version of the Stanford Health Assessment Questionnaire (HAQ) was used to measure daily life function.[4]

Laboratory analyses

Plasma and serum samples were stored at -70°C until assay. IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide 2 (anti-CCP) were analysed using enzyme immunoassay (Phadia 250, Thermofisher AB, Uppsala, Sweden). Levels of ≥ 5 international units (IU)/ml (IgM RF) and ≥ 7 arbitrary units (AU)/ml (anti-CCP) were regarded as positive. Samples from individual patients were analysed in parallel. When 100 healthy blood donor controls were analyzed in the same laboratory, 4 were IgM RF positive and none were anti-CCP positive, corresponding to 96% and 100% specificity, respectively.

Procollagen type I N-terminal propeptide (P1NP; marker of bone formation), C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (ICTP; both markers of bone degradation) were analysed as described earlier.[5]

Statistics

The SPSS V.21.0 statistical software was used. To test differences between groups, the Mann–Whitney U test or unpaired t test was used for continuous variables, whereas the Wilcoxon matched pairs test was used for paired comparisons and the χ^2 test for proportions.

To identify predictors of radiographic progression, univariate analyses of baseline clinical and demographic variables were performed. Variables with a p-value less than 0.1 were entered into multivariate logistic regression models with radiographic progression as the dependent variable. Prediction analyses in subgroups were justified by interaction analyses of treatment (prednisolone or no prednisolone) and anti-CCP (or RF) plus the interaction term between them (relevant interaction $p < 0.1$).

Results

Radiographic progression

After 2 years the frequency of patients with radiographic progression (progressors) was 26% in the P-group and 39% in the NoP-group, $p = 0.033$.

Baseline characteristics and associations between baseline variables and radiographic progression

Demographic and clinical characteristics at baseline in patients with and without progression of joint damage after 2 years are shown in table 1. Univariate analyses per treatment group showed that in the P-group progressors had significantly more swollen joints and higher TSS than non-progressors, whereas in the NoP-group presence of RF and anti-CCP as well as elevated CRP and TSS were associated with radiographic progression. The concentrations of P1NP, CTX-1 and ICTP did not differ significantly between progressors and non-progressors, irrespective of prednisolone treatment.

Table 1. Demographic and clinical characteristics at baseline separated into patients randomized to prednisolone (P-group, n=108) and no prednisolone (NoP-group, n=117) and further separated into those who after 2 years had progression in total Sharp score >5.8 or not, progressors and non-progressors, respectively.

Baseline characteristics	P-group			NoP-group		
	Progressors n=28	Non-progressors n=80	p-value	Progressors n=46	Non-progressors n=71	p-value
Age, years	50 (13)	52 (15)	0.57	58 (13)	58 (13)	0.89
Women, n (%)	17 (61)	52 (65)	0.68	29 (63)	46 (65)	0.85
Smokers						
ever, %	78.6	61.3	0.10	63.0	60.0	0.74
never, %	21.4	38.8		37.0	40.0	
Disease dur. mo	7 (3)	6 (4)	0.83	6 (3)	6 (3)	0.22
RF pos, n (%)	20 (74.1)	38 (54.3)	0.075	33 (86.8)	30 (48.4)	0.001
Anti-CCP pos, n (%)	20 (74.1)	41 (58.6)	0.157	31 (81.6)	28 (45.2)	0.001
DAS28	5.33 (1.34)	5.23 (1.02)	0.69	5.44 (1.06)	5.45 (0.98)	0.94
ESR, mm	41 (24)	36 (26)	0.38	43 (23)	34 (25)	0.06
Swollen joints, n	13 (5)	11 (5)	0.029	11 (6)	11 (5)	0.82
Tender joints, n	8 (7)	7 (5)	0.88	8 (7)	9 (6)	0.26
General health, VAS, mm	39 (29)	47 (21)	0.14	43 (23)	48 (24)	0.23
CRP (mg/L)	38 (31)	30 (30)	0.08 [†]	43 (38)	31 (37)	0.012[†]

Pain, VAS, mm	44 (25)	48 (22)	0.38	47 (20)	50 (22)	0.39
HAQ (0-3)	0.94 (0.71)	1.01 (0.53)	0.56	1.13 (0.58)	0.9 (69)	0.07
TSS	5.37 (6.11)	3.67 (10.16)	0.033[†]	8.50 (13.13)	2.53 (5.53)	0.001[†]
P1NP	33 (16)	22 (9)	0.074	48 (12)	49 (21)	0.82
CTX-1	0.26 (0.14)	5.1 (0.15)	0.15	0.35 (0.18)	0.33 (0.19)	0.82
1CTP	4.1 (1.8)	5.9 (7.7)	0.54	5.1 (1.4)	5.2 (2.6)	0.93

p- values represent differences between progressors and no progressors. Values are mean (SD). n = numbers; mo = months; Swollen and tender joints were calculated on 28 joints. VAS = visual analogue scale; TSS = Total Sharp score. P1NP = procollagen type I N-terminal propeptide, CTX-1 = C-terminal telopeptide crosslaps, 1CTP = C-terminal telopeptides of type I collagen. [†]Mann-Whitney U test.

Prednisolone and concomitant treatment

In the P-group, some patients reduced the prednisolone dose and 8 stopped treatment. In the NoP-group, 6 patients started prednisolone treatment during the study period. DMARD treatment (mostly methotrexate and sulphasalazine) was given to all patients and did not differ between progressors and non-progressors neither in the P-group nor in the NoP-group during the first three months.

Prediction of radiographic progression.

In addition to RF and anti-CCP, baseline swollen joint count, TSS, ESR, CRP and HAQ were univariately associated with radiographic progression (p<0.1) and were entered into multivariate logistic models, in which RF, anti-CCP and TSS proved to be independent predictors.

Prediction analyses in subgroups were justified by interaction analyses (relevant interaction p<0.1). Thus, relevant interactions between treatment and RF (p=0.061) and between treatment and anti-CCP (p=0.096) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), p=0.001, and OR (95%CI) 8.7 (2.5-31.3), p=0.001, respectively.

Change in RF and anti-CCP during two years follow-up

In both treatment groups most patients retained their RF and anti-CCP status (pos/neg) during the two study years; for the P-group, 82.3% and 87.5%, respectively, and for the

NoP-group, 88.9% and 98%, respectively. Some patients, however, reversed from RF and/or anti-CCP positivity to negativity; in the P-group 15.6% and 9.4%, respectively, and in the No-P-group 9.9% and 2.0%, respectively. More patients lost than acquired seropositivity.

RF and anti-CCP levels among seropositive patients did not differ between the treatment groups at baseline or at 2 years, but in both treatment groups there were significant reductions in both autoantibody levels during the study period (table 2). When calculated only on those patients who were compliant with the randomization and dose of prednisolone, the P-group had a larger reduction of anti-CCP than the NoP-group, $p=0.028$ (table 2).

Table 2. Levels of RF and anti-CCP (median (IQR)) in the patients positive for one or both of these antibodies in the two treatment groups.

	P-group (n=97)	NoP-group (n=100)	P-value between groups	P-value between groups, only patients with dose according to protocol (77 vs 94)
RF, baseline (IU/ml)	12.0 (1.3-58.0)	21.5 (1.9-80.5)	0.39	0.91
Anti-CCP, baseline (AU/ml)	28.0 (3.4-367.0)	43.5 (2.5-384.5)	0.63	0.38
RF, 2 years	4.1 (0.7-28.0)	9.5 (1.00-52.0)	0.14	0.41
Anti-CCP, 2 years	13.0 (2.3-141.0)	24.0(2.1-446.0)	0.70	1.00
Δ RF, 0-2 years	-1.1 (-20.3-0.20)**	-1.5 (-34.0-0)**	0.63	0.69
Δ Anti-CCP, 0-2 years	-1.9 (-55.4- -0.10) **	-0.3 (-88.0- 0.5)*	0.14	0.028

*= $p<0.05$, **= $p<0.001$ (Wilcoxon matched pairs test).

Discussion

The present study was undertaken to analyse if predictors for radiographic progression differed between early RA patients treated with or without prednisolone, in combination with DMARDs during the first two years after diagnosis. The main finding was that RF and anti-CCP predicted radiographic progression only in the group not treated with prednisolone.

The presence of RF and antibodies against citrullinated proteins/peptides (ACPA) has been found to predict the development of RA and also the severity of the disease, suggesting a possible pathogenic role for these autoantibodies.[6-8] If so, the present finding that RF- and anti-CCP-positivity did not predict radiographic progression in prednisolone treated patients may imply that prednisolone affects the pathogenic mechanisms associated with these antibodies in early RA. This possibility is in line with a role for RF in joint damage progression beyond its direct effect on disease activity.[9] Interestingly, such effects of RF, independent of disease activity, have been shown to be significantly associated only with progression of the erosion score, but not with the joint space narrowing score.[9] Similarly, we have earlier reported that the hampering effect of prednisolone on radiographic progression was valid only for erosions.[10]

The lack of association between autoantibody status and radiographic progression in the prednisolone treated patients is consistent with similar findings in patients treated with some biological agents.[11-12] It is further in line with the findings in the BEST study where the association of ACPA-status with joint damage progression was significantly more pronounced in patients treated with initial methotrexate monotherapy compared with those getting combination therapy with prednisolone or anti-TNF agents.[13] One explanation might be that early and intensive reduction of inflammation, also found here in the P-group, may suppress a strong autoimmune response.[14]

Such an explanation to the fact that the autoantibodies at baseline did not predict radiographic progression in the P-group is supported by the finding that more patients in this group reverted from seropositivity to negativity. In a recent study by Barra et al on early inflammatory arthritis, seroreversion occurred in rates similar to those in the present study without any influence on the prediction of outcome.[15] However, in another early

polyarthritis cohort the prognostic significance of initial RF and anti-CCP positivity was influenced by seroreversion of these antibodies.[16]

Not only antibody status but also serum level changes might be of importance in the prediction of outcome. Here we found that the levels of RF and anti-CCP decreased in both treatment groups. This decrease was significantly more profound in the P-group only if the calculation was based on the patients who strictly followed randomisation and dose. We suggest that such a subgroup analysis is important to find specific effects of prednisolone. Reports on the predictive value of changes in autoantibody levels are limited, but one study on early RA reports that changes in RF and ACPA levels were not associated with radiographic outcome.[17] In established RA, RF and ACPA level reductions are reported to be closely linked to treatment-associated improvements.[18] However, if such reductions are associated with structural changes remains unknown.

In conclusion, presence of RF and anti-CCP did not predict radiographic progression in patients treated with prednisolone in contrast to prednisolone-naïve patients. The data imply that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms. The clinical implication would be that the unfavourable prognosis associated with RF- and anti-CCP- positivity can be relieved by prednisolone treatment.

Acknowledgements

We thank research nurse Margareta Wörnert for excellent assistance, MD Kristina Albertsson for scoring radiographs and the following members of the BARFOT study group: Maria Andersson, Kristina Forslind, Catharina Keller, Ido Leden, Bengt Lindell, Ingemar Petersson, Christoffer Schaufelberger, Annika Teleman and Jan Theander.

Competing interest

None

Contributor statement

IH, AB, DvH and BS were involved in the original planning of the low-dose prednisolone study and interpretation of data. IH was responsible for data acquisition and preparing the manuscript, AB for scoring the radiographs and BS and DvH for statistical analyses. ILE

was involved in the statistical analyses and JR performed the analyses of RF and anti-CCP. All authors read and approved the manuscript.

Funding

This work was supported by grants from The Swedish Rheumatism Association, King Gustav V 80 year's Foundation, The Swedish Research Council, and through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet.

Data sharing statement

No additional data available.

References

1. Svensson B, Boonen A, Albertsson K, et al. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;**52**:3360-70.
2. van der Heijde DM, van Leeuwen MA, van Riel PL, et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;**35**:26-34.
3. Prevoo ML, van 't Hof MA, Kuper HH, et al. 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. Ekdahl C, Eberhardt K, Andersson SI, et al. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scand J Rheumatol* 1988;**17**:263-71.
5. Engvall IL, Svensson B, Tengstrand B, et al. Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. *Arthritis Res Ther* 2008;**10**:R128.
6. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2741-9.
7. van der Heijde DM, van Riel PL, van Leeuwen MA, et al. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. *British J Rheumatol* 1992;**31**:519-25.
8. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;**7**:R949-58.
9. Aletaha D, Alasti F, Smolen JS. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann Rheum Dis* 2013;**72**:875-80.
10. Engvall IL, Svensson B, Boonen A, et al. Low-dose prednisolone in early rheumatoid arthritis inhibits collagen type I degradation by matrix metalloproteinases as assessed by

serum ICTP--a possible mechanism for specific inhibition of radiological destruction. *Rheumatol* 2013;**52**:733-42.

11. Burmester GR, Feist E, Kellner H, et al. Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA). *Ann Rheum Dis* 2011;**70**:755-59.

12. Soto L, Sabugo F, Catalan D, et al. The presence of anti-citrullinated protein antibodies (ACPA) does not affect the clinical response to adalimumab in a group of RA patients with the tumor necrosis factor (TNF) alpha-308 G/G promoter polymorphism. *Clin Rheumatol* 2011;**30**:391-5.

13. van den Broek M, Dirven L, Klarenbeek NB, et al. The association of treatment response and joint damage with ACPA-status in recent-onset RA: a subanalysis of the 8-year follow-up of the BeSt study. *Ann Rheum Dis* 2012;**71**:245-8.

14. de Vries-Bouwstra JK, Goekoop-Ruiterman YPM, Verpoort KN, et al. Progression of joint damage in early rheumatoid arthritis. Association with HLA-DRB1, rheumatoid factor, and anti-citrullinated protein antibodies in relation to different treatment strategies. *Arthritis Rheum* 2008;**58**:1293-8.

15. Barra L, Bykerk V, Pope JE, et al. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;**40**:1259-67.

16. Guzman MC, Carrier N, Cossette P, et al. Outcomes in recent-onset inflammatory polyarthritis differ according to initial titers, persistence over time, and specificity of the autoantibodies. *Arthritis Care Res* 2010;**62**:1624-32.

17. Ursum J, Bos WH, van Dillen N, et al. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010;**12**:R8.

18. Bohler C, Radner H, Smolen JS, et al. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 2013;**72**:241-4.

Rheumatoid factor and anti-CCP do not predict progressive joint damage in patients with early rheumatoid arthritis treated with prednisolone – a randomized study

Ingjald Hafström, Inga-Lill Engvall, Johan Rönnelid, Annelies Boonen, Désirée van der Heijde, Björn Svensson on behalf of the BARFOT study group

Ingjald Hafström, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Inga-Lill Engvall, Rheumatology unit, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden;

Johan Rönnelid, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden;

Annelies Boonen, Department of Internal Medicine, Division of Rheumatology, University Hospital, and CAPHRI Research Institute Maastricht, The Netherlands;

Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands;

Björn Svensson, Department of Clinical Sciences, Section of Rheumatology, Lund University, Lund, Sweden.

Corresponding author: Ingjald Hafström, Rheumatology department, R92, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden. Telephone +46 8 58581831, fax +46 8 58587260; e-mail ingjald.hafstrom@karolinska.se

Keywords: Rheumatoid arthritis, RF, anti-CCP, radiographic progression, prednisolone

Word count: 1504

Abstract

Objective. To analyse if predictors of radiographic progression differ between patients treated with or without prednisolone in early rheumatoid arthritis (RA). Radiographs of hands and feet were assessed using the modified Sharp/van der Heijde score and radiographic progression was defined as an increase in total Sharp score above 5.8 (the smallest-detectable-change).

Design. Prospective, randomized study of patients with early RA.

Setting. Secondary level of care; six participating centres from southern Sweden; both urban and rural populations.

Participants. In all 225 patients, 64% women, with a diagnosis of RA according to the American College of Rheumatology criteria were included if they were between 18 and 80 years of age and had a disease duration of less than one year.

Intervention. The patients were randomised to 7.5 mg prednisolone daily for two years (P-group; n=108) or no prednisolone, (NoP-group; n=117) when they started with their first DMARD and were prospectively followed for two years.

Results. The frequency of patients with radiographic progression after two years was 26% in the P-group and 39% in the NoP-group (p=0.033). Relevant interactions between treatment and RF (p=0.061) and between treatment and anti-CCP (p=0.096) were found. RF and anti-CCP independently predicted radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), p=0.001, and OR (95%CI) 8.7 (2.5-31.3), p=0.001, respectively.

Conclusion. Presence of RF and anti-CCP predicted radiographic progression in patients not treated with prednisolone but failed to predict progression in patients treated with this drug. The data suggest that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms.

Trial registration: ISRCTN20612367

Strengths and limitation of this study

- A strength of the study is the prospective design with randomization of patients with early RA to treatment with low-dose prednisolone or no prednisolone together with disease modifying anti-rheumatic drug for two years.
- Another strength is that most patients followed the treatment they were randomized to.
- The main limitation is the rather small number of patients in each subgroup, which may reduce statistical power.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

• **Introduction**

Recent treatment strategies in early rheumatoid arthritis (RA) have considerably improved outcome. Nevertheless, most clinical trials as well as clinical practice show significant subgroups of patients who fail to respond and develop progressive joint damage.

In the BARFOT (Better Anti-Rheumatic Pharmacotherapy) low dose prednisolone study on 250 early (< 1 year disease duration) RA patients, joint damage progression was less frequent after two years in the group of patients who in addition to disease-modifying anti-rheumatic drugs (DMARDs) got prednisolone 7.5 mg daily compared to those treated with DMARDs alone.[1] Despite this achievement some patients in the prednisolone group deteriorated radiographically while some in the non-prednisolone group did not.

We therefore wanted to study if predictors of radiographic progression differed between patients treated with or without prednisolone in early RA.

Methods

Patients

The patients had all participated in the BARFOT low-dose prednisolone study in which radiographic progression was the primary outcome.[1] DMARDs were chosen by the treating physicians with the goal to achieve remission, defined as a Disease Activity Score (DAS28) <2.6. In addition, the patients were randomized to prednisolone, 7.5 mg/day, (P-group n=119) or no prednisolone (NoP-group n=131).

The present study population consisted of the 225 (90% of the randomized) patients who had radiographs of hands and feet at both baseline and the 2-year follow-up. Of these, 108 patients were in the P-group and 117 in the NoP-group.

All patients gave their informed consent and the ethics committees approved the study, which was performed in accordance with the Helsinki Declaration.

Radiographic assessment

Radiographs were scored for erosions, joint space narrowing and total Sharp scores (TSS) with known time sequence using the van der Heijde modification of the Sharp score, by two readers.[2] The smallest detectable change (SDC), based on interobserver data, was calculated to be 5.8 [1] admitting radiographic progressors to be defined as having a TSS >5.8.

Disease activity and physical function

Disease activity was assessed by DAS28.[3] The Swedish version of the Stanford Health Assessment Questionnaire (HAQ) was used to measure daily life function.[4]

Laboratory analyses

Plasma and serum samples were stored at -70°C until assay. IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide 2 (anti-CCP) were analysed using enzyme immunoassay (Phadia 250, Thermofisher AB, Uppsala, Sweden). Levels of ≥ 5 international units (IU)/ml (IgM RF) and ≥ 7 arbitrary units (AU)/ml (anti-CCP) were regarded as positive. Samples from individual patients were analysed in parallel. When 100 healthy blood donor controls were analyzed in the same laboratory, 4 were IgM RF positive and none were anti-CCP positive, corresponding to 96% and 100% specificity, respectively.

Procollagen type I N-terminal propeptide (P1NP; marker of bone formation), C-terminal telopeptide crosslaps (CTX-1) and C-terminal telopeptides of type I collagen (ICTP; both markers of bone degradation) were analysed as described earlier.[5]

Statistics

The SPSS V.21.0 statistical software was used. To test differences between groups, the Mann–Whitney U test, or unpaired t test was used for continuous variables, whereas the Wilcoxon matched pairs test was used for paired comparisons and the χ^2 test for proportions.

To identify predictors of radiographic progression, univariate analyses of baseline clinical and demographic variables were performed with $p < 0.10$ in univariate analyses were entered into multiple logistic regression models. Variables with a p-value less than 0.1 were entered into multivariate logistic regression models with radiographic progression as the dependent variable. Prediction analyses in subgroups were justified by interaction analyses of treatment (prednisolone or no prednisolone) and anti-CCP (or RF) plus the interaction term between them (relevant interaction $p < 0.1$).

~~To identify predictors of radiographic progression, baseline clinical and demographic variables with $p < 0.10$ in univariate analyses were entered into multiple logistic regression models. Prediction analyses in subgroups were justified by interaction analyses (relevant interaction $p < 0.1$).~~

Results

Radiographic progression

After 2 years the frequency of patients with radiographic progression (progressors) was 26% in the P-group and 39% in the NoP-group, p=0.033.

Baseline characteristics and associations between baseline variables and radiographic progression

Demographic and clinical characteristics at baseline in patients with and without progression of joint damage after 2 years are shown in table 1. Univariate analyses per treatment group showed that in the P-group progressors had significantly more swollen joints and higher TSS than non-progressors, whereas in the NoP-group presence of RF and anti-CCP as well as elevated CRP and TSS were associated with radiographic progression. The concentrations of P1NP, CTX-1 and ICTP did not differ significantly between progressors and non-progressors, irrespective of prednisolone treatment.

Table 1. Demographic and clinical characteristics at baseline separated into patients randomized to prednisolone (P-group, n=108) and no prednisolone (NoP-group, n=117) and further separated into those who after 2 years had progression in total Sharp score >5.8 or not, progressors and non- progressors, respectively.

Baseline characteristics	P-group			NoP-group		
	Progressors	Non-progressors	p-value	Progressors	Non-progressors	p-value
	n=28	n=80		n=46	n=71	
Age, years	50 (13)	52 (15)	0.57	58 (13)	58 (13)	0.89
Women, n (%)	17 (61)	52 (65)	0.68	29 (63)	46 (65)	0.85
Smokers						
ever, %	78.6	61.3	0.10	63.0	60.0	0.74
never, %	21.4	38.8		37.0	40.0	
Disease dur. mo	7 (3)	6 (4)	0.83	6 (3)	6 (3)	0.22
RF pos, n (%)	20 (74.1)	38 (54.3)	0.075	33 (86.8)	30 (48.4)	0.001
Anti-CCP pos, n (%)	20 (74.1)	41 (58.6)	0.157	31 (81.6)	28 (45.2)	0.001
DAS28	5.33 (1.34)	5.23 (1.02)	0.69	5.44 (1.06)	5.45 (0.98)	0.94
ESR, mm	41 (24)	36 (26)	0.38	43 (23)	34 (25)	0.06

Swollen joints, n	13 (5)	11 (5)	0.029	11 (6)	11 (5)	0.82
Tender joints, n	8 (7)	7 (5)	0.88	8 (7)	9 (6)	0.26
General health, VAS, mm	39 (29)	47 (21)	0.14	43 (23)	48 (24)	0.23
CRP (mg/L)	38 (31)	30 (30)	0.08 [†]	43 (38)	31 (37)	0.012[†]
Pain, VAS, mm	44 (25)	48 (22)	0.38	47 (20)	50 (22)	0.39
HAQ (0-3)	0.94 (0.71)	1.01 (0.53)	0.56	1.13 (0.58)	0.9 (69)	0.07
TSS	5.37 (6.11)	3.67 (10.16)	0.033[†]	8.50 (13.13)	2.53 (5.53)	0.001[†]
P1NP	33 (16)	22 (9)	0.074	48 (12)	49 (21)	0.82
CTX-1	0.26 (0.14)	5.1 (0.15)	0.15	0.35 (0.18)	0.33 (0.19)	0.82
1CTP	4.1 (1.8)	5.9 (7.7)	0.54	5.1 (1.4)	5.2 (2.6)	0.93

p- values represent differences between progressors and no progressors. Values are mean (SD). n = numbers; mo = months; Swollen and tender joints were calculated on 28 joints. VAS = visual analogue scale; TSS = Total Sharp score. P1NP = procollagen type I N-terminal propeptide, CTX-1 = C-terminal telopeptide crosslaps, 1CTP = C-terminal telopeptides of type I collagen. [†]Mann-Whitney U test.

Prednisolone and concomitant treatment

In the P-group, some patients reduced the prednisolone dose and 8 stopped treatment. In the NoP-group, 6 patients started prednisolone treatment during the study period. DMARD treatment (mostly methotrexate and sulphasalazine) was given to all patients and did not differ between progressors and non-progressors neither in the P-group nor in the NoP-group during the first three months.

Prediction of radiographic progression.

In addition to RF and anti-CCP, baseline swollen joint count, TSS, ESR, CRP and HAQ and TSS were univariately associated with radiographic progression ($p < 0.1$) and were entered into multivariate logistic models, in which RF, anti-CCP and TSS proved to be independent predictors.

Prediction analyses in subgroups were justified by interaction analyses (relevant interaction $p < 0.1$). Thus, rRelevant interactions between treatment and RF ($p = 0.061$) and between treatment and anti-CCP ($p = 0.096$) were found. RF and anti-CCP independently predicted

radiographic progression only in the NoP group, OR (95%CI) 9.4 (2.5-35.2), p=0.001, and OR (95%CI) 8.7 (2.5-31.3), p=0.001, respectively.

Change in RF and anti-CCP during two years follow-up

In both treatment groups most patients retained their RF and anti-CCP status (pos/neg) during the two study years; for the P-group, 82.3% and 87.5%, respectively, and for the NoP-group, 88.9% and 98%, respectively. Some patients, however, reversed from RF and/or anti-CCP positivity to negativity; in the P-group 15.6% and 9.4%, respectively, and in the No-P-group 9.9% and 2.0%, respectively. More patients lost than acquired seropositivity.

RF and anti-CCP levels among seropositive patients did not differ between the treatment groups at baseline or at 2 years, but in both treatment groups there were significant reductions in both autoantibody levels during the study period (table 2). When calculated only on those patients who were compliant with the randomization and dose of prednisolone, the P-group had a larger reduction of anti-CCP than the NoP-group, p=0.028 (table 2).

Table 2. Levels of RF and anti-CCP (median (IQR)) in the patients positive for one or both of these antibodies in the two treatment groups.

	P-group (n=97)	NoP-group (n=100)	P-value between groups	P-value between groups, only patients with dose according to protocol (77 vs 94)
RF, baseline (IU/ml)	12.0 (1.3-58.0)	21.5 (1.9-80.5)	0.39	0.91
Anti-CCP, baseline (AU/ml)	28.0 (3.4-367.0)	43.5 (2.5-384.5)	0.63	0.38
RF, 2 years	4.1 (0.7-28.0)	9.5 (1.00-52.0)	0.14	0.41
Anti-CCP, 2 years	13.0 (2.3-141.0)	24.0(2.1-446.0)	0.70	1.00

Δ RF, 0-2 years	-1.1 (-20.3-0.20)**	-1.5 (-34.0-0)**	0.63	0.69
Δ Anti-CCP, 0-2 years	-1.9 (-55.4- -0.10) **	-0.3 (-88.0-0.5)*	0.14	0.028

*= p<0.05, **= p<0.001 (Wilcoxon matched pairs test).

Discussion

The present study was undertaken to analyse if predictors for radiographic progression differed between early RA patients treated with or without prednisolone, in combination with DMARDs during the first two years after diagnosis. The main finding was that RF and anti-CCP predicted radiographic progression only in the group not treated with prednisolone.

The presence of RF and antibodies against citrullinated proteins/peptides (ACPA) has been found to predict the development of RA and also the severity of the disease, suggesting a possible pathogenic role for these autoantibodies.[6-8] If so, the present finding that RF- and anti-CCP-positivity did not predict radiographic progression in prednisolone treated patients may imply that prednisolone affects the pathogenic mechanisms associated with these antibodies in early RA. This possibility is in line with a role for RF in joint damage progression beyond its direct effect on disease activity.[9] Interestingly, such effects of RF, independent of disease activity, have been shown to be significantly associated only with progression of the erosion score, but not with the joint space narrowing score.[9] Similarly, we have earlier reported that the hampering effect of prednisolone on radiographic progression was valid only for erosions.[10]

The lack of association between autoantibody status and radiographic progression in the prednisolone treated patients is consistent with similar findings in patients treated with some biological agents.[11-12] It is further in line with the findings in the BEST study where the association of ACPA-status with joint damage progression was significantly more pronounced in patients treated with initial methotrexate monotherapy compared with those getting combination therapy with prednisolone or anti-TNF agents.[13] One explanation

might be that early and intensive reduction of inflammation, also found here in the P-group, may suppress a strong autoimmune response.[14]

Such an explanation to the fact that the autoantibodies at baseline did not predict radiographic progression in the P-group is supported by the finding that more patients in this group reverted from seropositivity to negativity. In a recent study by Barra et al on early inflammatory arthritis, seroreversion occurred in rates similar to those in the present study without any influence on the prediction of outcome.[15] However, in another early polyarthritis cohort the prognostic significance of initial RF and anti-CCP positivity was influenced by seroreversion of these antibodies.[16]

Not only antibody status but also serum level changes might be of importance in the prediction of outcome. Here we found that the levels of RF and anti-CCP decreased in both treatment groups. This decrease was significantly more profound in the P-group only if the calculation was based on the patients who strictly followed randomisation and dose. We suggest that such a subgroup analysis is important to find specific effects of prednisolone. Reports on the predictive value of changes in autoantibody levels are limited, but one study on early RA reports that changes in RF and ACPA levels were not associated with radiographic outcome.[17] In established RA, RF and ACPA level reductions are reported to be closely linked to treatment-associated improvements.[18] However, if such reductions are associated with structural changes remains unknown.

In conclusion, presence of RF and anti-CCP did not predict radiographic progression in patients treated with prednisolone in contrast to prednisolone-naïve patients. The data imply that early treatment with prednisolone may modulate not only inflammation but also autoimmunity-associated pathogenetic mechanisms. The clinical implication would be that the unfavourable prognosis associated with RF- and anti-CCP- positivity can be relieved by prednisolone treatment.

Acknowledgements

We thank research nurse Margareta Wörnert for excellent assistance, MD Kristina Albertsson for scoring radiographs and the following members of the BARFOT study group: Maria Andersson, Kristina Forslind, Catharina Keller, Ido Leden, Bengt Lindell, Ingemar Petersson, Christoffer Schaufelberger, Annika Teleman and Jan Theander.

Competing interest

None

Contributor statement

IH, AB, DvH and BS were involved in the original planning of the low-dose prednisolone study and interpretation of data. IH was responsible for data acquisition and preparing the manuscript, AB for scoring the radiographs and BS and DvH for statistical analyses. ILE was involved in the statistical analyses and JR performed the analyses of RF and anti-CCP. All authors read and approved the manuscript.

Funding

This work was supported by grants from The Swedish Rheumatism Association, King Gustav V 80 year's Foundation, The Swedish Research Council, and through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet.

Data sharing statement

There is no additional data available.

References

1. Svensson B, Boonen A, Albertsson K, et al. Low-dose prednisolone in addition to the initial disease-modifying antirheumatic drug in patients with early active rheumatoid arthritis reduces joint destruction and increases the remission rate: a two-year randomized trial. *Arthritis Rheum* 2005;**52**:3360-70.

2. van der Heijde DM, van Leeuwen MA, van Riel PL, et al. Biannual radiographic assessments of hands and feet in a three-year prospective followup of patients with early rheumatoid arthritis. *Arthritis Rheum* 1992;**35**:26-34.

3. Prevoo ML, van 't Hof MA, Kuper HH, et al. 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. Ekdahl C, Eberhardt K, Andersson SI, et al. Assessing disability in patients with rheumatoid arthritis. Use of a Swedish version of the Stanford Health Assessment Questionnaire. *Scand J Rheumatol* 1988;**17**:263-71.

5. Engvall IL, Svensson B, Tengstrand B, et al. Impact of low-dose prednisolone on bone synthesis and resorption in early rheumatoid arthritis: experiences from a two-year randomized study. *Arthritis Res Ther* 2008;**10**:R128.

6. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2741-9.

7. van der Heijde DM, van Riel PL, van Leeuwen MA, et al. Prognostic factors for radiographic damage and physical disability in early rheumatoid arthritis. A prospective follow-up study of 147 patients. *British J Rheumatol* 1992;**31**:519-25.

8. van der Helm-van Mil AH, Verpoort KN, Breedveld FC, et al. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res Ther* 2005;**7**:R949-58.

9. Aletaha D, Alasti F, Smolen JS. Rheumatoid factor determines structural progression of rheumatoid arthritis dependent and independent of disease activity. *Ann Rheum Dis* 2013;**72**:875-80.

10. Engvall IL, Svensson B, Boonen A, et al. Low-dose prednisolone in early rheumatoid arthritis inhibits collagen type I degradation by matrix metalloproteinases as assessed by

serum ICTP--a possible mechanism for specific inhibition of radiological destruction.

Rheumatol 2013;**52**:733-42.

11. Burmester GR, Feist E, Kellner H, et al. Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA). *Ann Rheum Dis* 2011;**70**:755-59.

12. Soto L, Sabugo F, Catalan D, et al. The presence of anti-citrullinated protein antibodies (ACPA) does not affect the clinical response to adalimumab in a group of RA patients with the tumor necrosis factor (TNF) alpha-308 G/G promoter polymorphism. *Clin Rheumatol* 2011;**30**:391-5.

13. van den Broek M, Dirven L, Klarenbeek NB, et al. The association of treatment response and joint damage with ACPA-status in recent-onset RA: a subanalysis of the 8-year follow-up of the BeSt study. *Ann Rheum Dis* 2012;**71**:245-8.

14. de Vries-Bouwstra JK, Goekoop-Ruiterman YPM, Verpoort KN, et al. Progression of joint damage in early rheumatoid arthritis. Association with HLA-DRB1, rheumatoid factor, and anti-citrullinated protein antibodies in relation to different treatment strategies. *Arthritis Rheum* 2008;**58**:1293-8.

15. Barra L, Bykerk V, Pope JE, et al. Anticitrullinated protein antibodies and rheumatoid factor fluctuate in early inflammatory arthritis and do not predict clinical outcomes. *J Rheumatol* 2013;**40**:1259-67.

16. Guzman MC, Carrier N, Cossette P, et al. Outcomes in recent-onset inflammatory polyarthritis differ according to initial titers, persistence over time, and specificity of the autoantibodies. *Arthritis Care Res* 2010;**62**:1624-32.

17. Ursum J, Bos WH, van Dillen N, et al. Levels of anti-citrullinated protein antibodies and IgM rheumatoid factor are not associated with outcome in early arthritis patients: a cohort study. *Arthritis Res Ther* 2010;**12**:R8.

18. Bohler C, Radner H, Smolen JS, et al. Serological changes in the course of traditional and biological disease modifying therapy of rheumatoid arthritis. *Ann Rheum Dis* 2013;**72**:241-4.



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4, ref 1
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/a
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4, ref 1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4, ref 1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4
	6b	Any changes to trial outcomes after the trial commenced, with reasons	4
Sample size	7a	How sample size was determined	Power calculation
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Ref 1
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Ref 1
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Ref1

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Ref 1
	11b	If relevant, description of the similarity of interventions	na
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	page
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Ref 1
	14b	Why the trial ended or was stopped	Ref 1
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Ref 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Ref 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Ref 1
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	na
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	na
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Ref 1
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	2
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	9-10
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	9
Other information			
Registration	23	Registration number and name of trial registry	ISRCTN2061 2367
Protocol	24	Where the full trial protocol can be accessed, if available	na
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	10

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

For peer review only