MS. JOSEPHINE AMKREUTZ (Orcid ID : 0000-0001-5636-9198)

DR. EMMA CORINE DE MOEL (Orcid ID : 0000-0002-8940-8330)

DR. CARL TURESSON (Orcid ID : 0000-0002-3805-2290)

DR. DIANE VAN DER WOUDE (Orcid ID : 0000-0001-8121-5879)

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The relation between autoantibodies and bone mineral density in patients with rheumatoid arthritis

Josephine A.M.P. Amkreutz[1] BSc, Emma C. de Moel[1] MD, Lisa Theander[2] MD PhD, Minna Willim[3,4], Lotte Heimans[5] MD PhD, Jan-Åke Nilsson[2,3] BSc, Magnus K. Karlsson[6,7] MD PhD, Tom W.J. Huizinga[1] MD PhD, Kristina E. Åkesson[6,7] MD PhD, Lennart T.H. Jacobsson[2,8] MD PhD, Cornelia F. Allaart[1] MD PhD, Carl Turesson[2,3] MD PhD, Diane van der Woude[1] MD PhD

[1] Department of Rheumatology, Leiden University Medical Center, Leiden, the Netherlands

[2] Rheumatology, Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden

[3] Department of Rheumatology, Skåne University Hospital, Malmö and Lund, Sweden

[4] Rheumatology, Department of Clinical Sciences, Lund, Lund University, Lund, Sweden

[5] Department of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

[6] Clinical and Molecular Osteoporosis Research Unit, Department of Clinical Sciences, Lund

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University, Malmö, Sweden
[7] Department of Orthopedics, Skåne University Hospital, Malmö, Sweden
[8] Department of Rheumatology and Inflammation Research, Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden

Corresponding author:
Diane van der Woude, MD PhD, Department of Rheumatology
Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands
E-mail: dvanderwoude@lumc.nl
Telephone: +31 71 526 34 23
Fax: +31 71 526 67 52

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ABSTRACT

Objective

Autoantibodies such as anti-citrullinated protein antibodies (ACPA) have been described to induce bone loss in RA, which could also be reflected in bone mineral density (BMD). We therefore examined the association between autoantibodies and osteoporosis in two independent RA-cohorts.

Methods

Dual X-ray absorptiometry (DXA) of lumbar spine (LS) and left hip (LH) was performed in 408 Dutch and 198 Swedish early RA-patients during five and ten years respectively. The longitudinal effect of ACPA and other autoantibodies on several BMD measures was studied using generalized estimating equations.

Results

In the Dutch cohort, ACPA-positive patients had a significantly lower baseline BMD compared to ACPA-negative patients (LH: Estimated Marginal Means (Confidence Interval): 0.92 (0.91-0.93) versus 0.95 (0.03-0.97) g/cm^2 (p=0.01)). In accordance, significantly lower baseline Z-scores were observed in the ACPA-positive group compared to the ACPA-negative group (LH: 0.18 (0.08-0.29) vs 0.48 (0.33-0.63) (p<0.01)). However, despite clear baseline differences, ACPA-positivity was not associated with greater decrease in absolute BMD or Z-score over time. Furthermore, there was no association between BMD and higher ACPA levels or other autoantibodies (RF and anti-CarP). In the Swedish cohort, ACPA-positive patients tended to have a higher baseline prevalence of osteopenia (p=0.04), but again, ACPA-positivity was not associated with more osteopenia or osteoporosis over time.

Conclusion

The presence of ACPA is associated with a significantly lower baseline BMD, but not with greater BMD loss over time in treated RA-patients. These results suggest that ACPA alone do not appear to contribute to bone loss after disease onset when disease activity is well managed.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by polyarthritis and an increased risk of osteoporosis[1]. It is known that patients with RA have twice the risk of osteoporosis-related fracture compared to age-matched controls, associating with high morbidity and mortality[2]. Although some of the mechanisms leading to bone loss in RA have been clarified (such as the effect of cytokines), the precise relationship between the immunopathogenesis of RA (e.g. autoantibodies) and osteoporosis remains unclear.

One of the most important serological markers in RA is the presence of anti-citrullinated protein antibodies (ACPA), which is a well-known predictive marker for a more destructive disease course[3]. ACPA may affect systemic bone mineral density (BMD) loss, since seropositive patients (especially those with higher levels) have been described to have lower systemic BMD and more osteoporosis[4-6].

There are two hypotheses for how ACPA might affect BMD: that ACPA represent a unique type of antibody able to directly induce bone loss, or that ACPA mediate bone loss only in the presence of concomitant inflammation.

Regarding the first hypothesis, some data suggest that ACPA can bind to and activate osteoclasts[7,8], which leads to increased osteoclast-mediated bone degradation and elevated serum levels of collagen degradation products such as RANKL[9]. This process is believed to occur independently of inflammatory status[6,10], since bone remodelling starts even before the onset of clinical disease[11]. In addition, altered bone metabolism has been observed in

healthy subjects with ACPA[12] and ACPA initiate bone loss when injected into mice[7], further supporting a possible direct pathogenic link between ACPA and bone destruction in RA.

However, (chronic) inflammation alone could also lead to bone degradation in RA via osteoclast activation mediated by pro-inflammatory cytokines[13,14]. ACPA could therefore characterize a particular subset of RA with a more inflammatory profile that in turn could result in more bone loss. This hypothesis is supported by preliminary studies indicating that RA-patients with a higher disease activity and higher levels of inflammatory markers suffer from more bone loss[15]. Lower BMD values in ACPA-positive patients can also be attributed to more aggressive prednisone bridging in ACPA-positive patients, which in itself is a risk factor for bone loss[16].

Longitudinal data, including detailed information about disease activity and treatment with DMARDs and glucocorticoids is necessary to elucidate the exact association between ACPA and bone loss in RA, which could provide clues about the underlying biological mechanisms. We therefore performed an in-depth investigation into the relation between autoantibodies and BMD by examining yearly DXA scores in two independent cohorts of RA-patients.

PATIENTS AND METHODS

Study design and patient selection

We used data from two large RA-cohorts that were analysed separately. The Dutch Induction therapy with Methotrexate and Prednisone in Rheumatoid Or Very Early arthritic Disease (IMPROVED) study is a multicentre, randomized controlled trial in which 610 patients with early (symptom duration <2 years) untreated RA or undifferentiated arthritis (UA) received remission (DAS<1.6) steered treatment between 2007 and 2010. For the Swedish cohort, 233 consecutive patients with early RA (symptom duration <12 months), recruited between

1995 and 2005 in the area of the city of Malmö, were followed according to a structured programme. Detailed in- and exclusion criteria as well as the exact study protocols have been described previously[17,18]. For both studies, ethical permission was granted and written informed consent was obtained from all patients.

At baseline, ACPA (anti-CCP2) IgG and Rheumatoid Factor (RF) IgM were measured by standard clinical methods. In the Dutch cohort, antibodies directed against carbamylated antigens (anti-CarP) were analysed by validated in-house assay as described previously[19]. RA was classified according to the ACR/EULAR 2010 RA-criteria in the Dutch cohort[20] and the ACR 1987 criteria[21] in the Swedish cohort. Data from RA-patients aged 20 years and older with a known ACPA-status were used for this study, resulting in 408 Dutch patients and 198 Swedish patients. Out of the 408 Dutch RA-patients, a subgroup of 128 patients with a relatively high disease activity (mean DAS>1.8 during the first two years after inclusion) was selected for separate analyses to assess the association between ACPA and BMD in the presence of more inflammation.

BMD measurements

BMD was assessed by dual-energy X-ray absorptiometry (DXA). In the Dutch cohort DXA scans were performed at the left total hip and first to fourth (L1-L4) or second to fourth (L2-L4) lumbar spine vertebrae every year for five years. For the Swedish cohort, DXA scans at the left femoral neck and second to fourth (L2-L4) lumbar spine vertebrae were obtained at inclusion and after 2, 5 and 10 years. BMD was expressed as absolute values (in g/cm^2), T-scores (in standard deviations (SD) from the mean of healthy young adults) or Z-scores (in SDs above or below the mean of a control population matched on age, sex and ethnicity)[22]. Osteopenia was defined as -2.5<T-score≤-1.0 at any location and osteoporosis was defined as a T-score≤-2.5 at any location. Dutch centres used the Hologic densitometer system, whereas Swedish data derived from the Lunar densitometer system. For the Dutch cohort, lumbar scores were determined according to the Hologic Spine reference group and femoral scores according to the National Health and Nutrition Examination Survey femur reference population[23]. BMD scores for the Swedish cohort were calculated using a cohort of healthy

individuals (146 men and 178 women, age 20-87) from the same area as the reference population[24].

Statistical analysis

First we performed univariate analyses to determine which of the covariates should be included in the final models. Variables that were univariably associated with ACPA-status and one of the outcomes of interest ($p \leq 0.1$) in at least one of the cohorts were included as covariates in the final models of both cohorts, namely: sex, age, BMI, symptom duration, smoking status and serum 25-OH vitamin D levels. Furthermore, the following variables were added to the models based on literature and a priori hypotheses: prednisone usage, DAS44, HAQ and CRP levels. The association between ACPA and BMD over time was modelled using generalized estimating equations (GEE), which allow for missing data in the outcome and account for clinical and demographic factors that differ between the two groups. With repeated measurements of BMD scores as the dependent variable, we investigated whether ACPA-status was associated with changes in BMD. The same was done for osteopenia- or osteoporosis prevalence. An interaction term of ACPA-status*time was added to determine whether yearly changes in the outcome variables were different between ACPA-positive and ACPA-negative patients. The final models were adjusted for the following baseline variables: age, sex, BMI, symptom duration and smoking status, as well as the following longitudinal time-varying measurements: disease activity (as assessed by DAS44), prednisone intake, Health Assessment Questionnaire Disability Index (HAQ), C-reactive protein levels (CRP levels) and serum 25-OH vitamin D levels (vitamin D levels only available for the Dutch cohort). Since there was no difference in the intake of anti-osteoporotic medication (bisphosphonates, vitamin D or calcium supplementation) between ACPA-positive versus ACPA-negative patients, these were not included in the final analyses. Due to missingness of data, multiple imputation by chained equations (MICE) with predictive mean matching on five nearest neighbours was used to create 20 imputed datasets. All data of variables considered relevant for BMD were included. For the analyses conducted on these 20 imputed datasets, only results after imputation are reported, which did not differ from the results obtained before imputation. All Dutch statistical analyses were performed using STATA 14SE and all Swedish analyses were performed using IBM SPSS

Statistics, Version 26. P-values ≤0.05 were considered significant. Holmes-Bonferroni method was used to correct the alpha level for multiple testing.

RESULTS

Patient characteristics

The baseline characteristics of all patients included in this study are displayed in Table 1. The only notable differences in demographic or clinical variables between ACPA-positive and ACPA-negative patients were DAS, HAQ and BMI for the Dutch cohort and CRP levels for the Swedish cohort. The higher disease activity measures in the Dutch ACPA-negative group can be explained by the use of ACR/EULAR 2010 criteria for RA, which require ACPA-negative patients to have more joint involvement and higher acute phase reactants to fulfil the definition of RA. A higher body mass index among Dutch ACPA-negative patients is in line with previous findings[25], as well as an association between ACPA and smoking[26] and ACPA and CRP respectively[27] in the Swedish cohort.

Patient characteristics and treatment at follow-up visits are shown in Supplementary Table 1. The usage of conventional synthetic DMARDs (csDMARDs), biological DMARDs (bDMARDs) and prednisone at later time points was generally lower among ACPA-negative patients, as expected based on previous results of the IMPROVED study showing a higher achievement of drug-free remission (DFR) in this subset of patients[28].

ACPA-positive patients have lower baseline BMD values

In the Dutch (NLD) cohort, ACPA-positive patients had a significantly lower baseline absolute BMD than ACPA-negative patients (Figure 1A+B). The same was observed for Z-scores (Supplementary Figure 1). For the Swedish (SWE) cohort, ACPA-positive patients also had slightly lower BMD values at baseline, but the difference was far less pronounced than in the Dutch cohort and did not reach statistical significance (Figure 1C+D). Of note, no

conclusions can be drawn from statistical comparisons between the two cohorts, since the Dutch and Swedish data were analysed in separate models.

The association between ACPA-status and BMD measures at baseline and over time was analysed using GEE, the results of which are shown in Table 2. We found that ACPA-positivity was significantly associated with lower baseline absolute BMD values in the Dutch cohort, both at the lumbar spine (p=0.03) and the left hip (p=0.01). Baseline Z-scores were also significantly lower at both locations in the ACPA-positive group. Differences in the Swedish cohort were not significant, although point estimates for the ACPA-positive subset were slightly lower than for the ACPA-negative subset at both measurement sites. When the final analyses for the Dutch and the Swedish cohort were adjusted for longitudinal intake of anti-osteoporotic medication, the results did not change (Supplementary Table 2).

Given the possible negative influence of ACPA on BMD, we expected the prevalence of osteopenia or osteoporosis to be higher among ACPA-positive patients compared to ACPA-negative patients. This was indeed the case in the Swedish cohort, where a significantly higher baseline prevalence of osteopenia was found for the ACPA-positive patients (p=0.04) (Table 2). The prevalence of osteoporosis at baseline however, did not differ between the two groups. In the Dutch cohort on the other hand, there was no association between ACPA-positivity and a higher prevalence of osteopenia or osteoporosis at baseline.

In total, ACPA-positive patients appear to have slightly lower BMD values at baseline in both cohorts, but the BMD measurements in which this is reflected, are different (NLD: absolute BMD and Z-score, SWE: osteopenia). Although not all differences were significant after correction for multiple testing, ACPA-positive patients overall had slightly lower BMD values at baseline in both cohorts.

ACPA-positivity is not associated with more BMD loss over time

We hypothesized that ACPA-positive patients would have a greater decline in BMD over time compared to ACPA-negative patients. However, in contrast to the baseline differences, we found no association between ACPA-status and yearly changes in BMD, as shown in Figure 1

and Table 2. ACPA-positivity was not associated with a significantly greater decline in absolute BMD values during the follow-up periods of 5 years (NLD) or 10 years (SWE) at either location. Also with regard to osteopenia or osteoporosis, ACPA was not associated with an increase over time in either cohort. In accordance, also changes in Z-scores over time did not differ between the two groups at either measurement site (Supplementary Figure 1).

There is no association between ACPA levels and BMD

To investigate whether higher levels of ACPA are associated with greater BMD loss, we analysed the association between ACPA IgG levels at inclusion and longitudinal BMD scores. At baseline, we found that higher levels of ACPA were not significantly associated with a lower BMD (Table 3). This was observed for absolute BMD values as well as for Z-scores, at both lumbar and femoral sites. There was also no association between higher levels of ACPA IgG at baseline and more absolute BMD loss over time (Supplementary Figure 2).

Other autoantibodies are not independently associated with BMD

In light of the associations we found between ACPA and baseline BMD, we extended our analyses in the Dutch cohort to other autoantibodies associated with RA: RF and anti-CarP. Table 4 lists the differences in BMD measures between seropositive versus seronegative patients for the different autoantibodies. We found that RF-positive patients had a lower baseline BMD compared to RF-negative patients (lumbar spine, p=0.04). The same was found for anti-CarP (left hip, p=0.04). Since both RF and anti-CarP frequently co-occur with ACPA, we adjusted the analyses for ACPA, after which both RF and anti-CarP were no longer associated with lower baseline BMD scores at any given locations. In contrast, the association between ACPA and lower baseline BMD values at the left hip remained significant after correction for the presence of RF and anti-CarP. In accordance with the previously described results for ACPA, no association was found between RF-positivity or anti-CarP-positivity and more decline in BMD over time. Finally, there was no baseline or longitudinal association between the quantitative number of autoantibodies present in a patient (ACPA, RF, anti-CarP: 0-3) and (loss of) BMD either at baseline or over time.

In summary, the association between autoantibody presence and lower baseline BMD appears to be most clear for ACPA, independent of the presence of other autoantibodies.

There is no association between ACPA and BMD in patients with a high disease activity

Inflammation is hypothesized to play a role in BMD loss in RA[15]. This raises the question whether the lack of association observed between ACPA and BMD loss over time could be due to the fact that there was very little disease activity and thus inflammation over time, especially in the Dutch patients treated with a treat-to-target approach with a DAS-target<1.6. Perhaps an association between ACPA and BMD loss over time would have been apparent in the setting of higher levels of inflammation/disease activity. To investigate this, we attempted to identify a subgroup of patients with higher disease activity in the Dutch cohort. In light of the overall very low disease activity in this cohort, we defined this higher-disease activity group as having a mean DAS>1.8 during the first two years after inclusion (baseline visit not included). In this subgroup of 128 patients, again no association was found between ACPA and absolute BMD values at baseline at the given sites (Figure 2A+B). In line with the results obtained from data of all patients included in the study (regardless of DAS), no association was found between ACPA and more bone loss over time.

DISCUSSION

This is the first study, to the best of our knowledge, investigating the important link between ACPA and BMD in a longitudinal manner in early untreated RA-patients. In this study, we found that ACPA are associated with lower systemic BMD at disease onset. This was particularly the case at femoral sites, where the observed values remained significant after correction for multiple testing. However, in spite of differences in baseline BMD between ACPA-positive and ACPA-negative patients, ACPA-positivity does not associate with greater BMD loss over time in patients receiving standard clinical care or a tight remission-steered treatment. Finally, there is no association between BMD and other RA-specific autoantibodies (such as RF and anti-CarP), nor between BMD and the number of autoantibodies in a patient.

Our results align with previous findings showing lower baseline BMD values amongst ACPA-positive compared to ACPA-negative patients. Moreover, this study is of important additive value, since it provides new insights into the course of BMD loss over time in patients with RA. Although no longitudinal differences were observed between the two groups, baseline differences were pronounced. Considering these results, it might be unlikely that the mere presence of ACPA is sufficient to cause bone loss in RA, since ACPA remain present after the start of treatment, yet ACPA-positive patients do not exhibit more bone loss than ACPA-negative patients. Our results therefore suggest alternative explanations than previous findings which supported the theory that ACPA induce bone loss independently of inflammatory status by directly binding to osteoclasts, stimulating osteoclast differentiation and proliferation.

Instead, lower BMD in ACPA-positive patients could possibly be an effect of inflammation. This hypothesis is supported by preliminary studies indicating that adequate suppression of disease activity and thus inflammation is key to prevent further bone loss and thereby stabilize BMD in patients with RA[13,29]. Furthermore, it has been suggested that suppression

of inflammation effectively prevents bone loss in ACPA-positive and ACPA-negative patients in equal measure. Earlier studies have demonstrated that inhibition of IL-8 interferes with osteoclastogenesis and thus prevents osteolysis[30,31]. Moreover, ACPA are only associated with higher erosion scores in the clinically suspect arthralgia stage of RA when concomitant inflammation is present, indicating that inflammation functions as a key mediator in the link between ACPA and erosion development[32]. Since there is strong evidence that erosive disease and systemic BMD loss in RA have common pathways in their pathogenesis[33,34], these results might also suggest an indirect association between ACPA and bone loss via inflammation.

In the current study, we found a stronger association between BMD and ACPA than between BMD and RF or anti-CarP. This could be a reflection of the fact that ACPA seem to represent a type of antibody that is able to define a particular subset of patients with RA more discriminately than RF or anti-CarP, based on for example specific associations with certain genetic and environmental risk factors[35]. This specific subset of RA-patients might also tend to suffer from more severe bone loss. In contrast to the findings by Orsolini et al.[5], we found no level-dependent effect of ACPA on baseline BMD.

Our study has several limitations. One is that we do not know the natural course of BMD over time in the absence of therapeutic intervention. We cannot exclude that ACPA might have been associated with BMD loss over time if patients had not been treated. However, this limitation is unavoidable in modern RA research, because all patients are treated. This limitation could also be seen as an advantage, since it afforded us the opportunity to assess the effect of autoantibody presence in the setting of optimal control of disease activity. Furthermore, anti-osteoporotic treatment, which was in part initiated based on the DXA results in the study, may have prevented further BMD loss during follow-up. Although this could theoretically have affected our comparisons, we have no indication that anti-osteoporotic medication was preferentially prescribed to ACPA-positive or ACPA-negative patients. Another limitation is that we cannot exclude that our lumbar spine DXAs are sensitive to increasing degenerative and osteoarthritic changes associated with ageing. This

could explain why lumbar BMD measurement showed a very slight increase over time. Furthermore, differences regarding absolute BMD values and Z-scores between ACPA-positive and ACPA-negative patients in the Dutch cohort were not exactly replicated in the Swedish cohort. This could be due to the fact that there were fewer Swedish patients, resulting in less power to detect differences. Finally, despite the clear statistically significant baseline differences, absolute differences in mean BMD measures between ACPA-positive and ACPA-negative patients were minor, meaning the clinical relevance of these findings has yet to be established.

Our study also has several strengths, such as the use of two independent cohorts with large sample sizes. Because of the long follow-up periods of 5 and 10 years respectively, we were able to not only investigate the link between autoantibodies and BMD on a baseline level, but also to determine the impact of these autoantibodies on long-term changes in BMD while accounting for various relevant covariates. By selecting patients diagnosed with early untreated arthritis, we were able to study the effect of autoantibodies on BMD without prior confounding by therapy.

In conclusion, we found that ACPA-positive patients have a significantly lower baseline BMD compared to ACPA-negative patients. However, ACPA-positivity is not associated with more bone loss over time in early RA-patients who are treated according to modern strategies. These results indicate that ACPA alone do not seem to contribute to bone loss after the onset of clinical disease in the absence of severe inflammation.

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The Netherlands		Lumbar spine				Left hip (total hip)		
		ACPA-positive	ACPA-negative	p-value		ACPA-positive	ACPA-negative	p-value
		The Netherlands				Sweden		
		ACPA-positive (n=268)	ACPA-negative (n=140)	p-value		ACPA-positive (n=114)	ACPA-negative (n=84)	p-value
Age, years mean (SD)		52 (13)	54 (14)	0.27		61 (12)	62 (16)	0.78
Female n (%)		188 (70)	92 (66)	0.36		81 (71)	61 (73)	0.81
BMI mean (SD)		25.6 (4.3)	26.6 (4.9)	**0.02**		25.4 (4.1)	24.9 (3.9)	0.36
Smoking status, n (%)				0.09				**0.01**
	Never	151 (57)	90 (65)		Never	25 (22)	34 (42)	
	Ever	116 (43)	48 (35)		Former	40 (36)	25 (31)	
					Current	47(42)	22 (27)	
Symptom duration, weeks median (IQR)		18 (9-36)	14 (9-28)	0.18		35 (26-44)	31 (22-43)	0.11
CRP, mg/L median (IQR)		13 (6-29)	11 (4-29)	0.32		10 (<9-32)	<9 (<9-17)	**0.05**
DAS mean (SD)		3.3 (0.9)	3.6 (0.9)	**<0.01**		3.3 (1.2)	3.2 (1.1)	0.48
HAQ mean (SD)		1.1 (0.7)	1.3 (0.7)	**0.02**		0.8 (0.6)	0.9 (0.7)	0.29
Calcium intake, mg/day mean (SD)		822 (281)	870 (327)	0.13		Not available	Not available	.
Serum 25-OH vitamin D, nmol/L mean (SD)		61 (30)	55 (27)	0.06		Not available	Not available	.

TABLES AND FIGURES

Table 1. Baseline characteristics of the study population from the Netherlands (n=408) and Sweden (n=198). P-values are based on t-tests, Mann-Whitney U tests or Chi-squared tests for normally distributed, non-normally distributed and dichotomous variables respectively.

Absolute BMD (g/cm²)	**Baseline EMM (95% CI)**	1.01 (1.00 to 1.03)	1.05 (1.02 to 1.08)	**0.03**	0.92 (0.91 to 0.93)	0.95 (0.93 to 0.97)	**0.01***
	Yearly change β (95% CI)	-0.002 (-0.004 to 0.001)	0.0004 (-0.004 to 0.004)	0.61	-0.003 (-0.006 to -0.001)	-0.004 (-0.008 to 0.00002)	0.89
Z-score	**Baseline EMM (95% CI)**	0.32 (0.16 to 0.47)	0.62 (0.38 to 0.86)	**0.04**	0.18 (0.08 to 0.29)	0.48 (0.33 to 0.63)	**<0.01***
	Yearly change β (95% CI)	0.038 (0.017 to 0.058)	0.060 (0.027 to 0.094)	0.43	0.008 (-0.007 to 0.023)	0.001 (-0.022 to 0.025)	0.37
Osteopenia	**Baseline n (%)**	94 (38.4)	50 (37.6)	0.48			
	5 years n (%)	77 (43.8)	31 (38.8)	0.65†			
Osteoporosis	**Baseline n (%)**	21 (8.6)	9 (6.8)	0.33			
	5 years n (%)	14 (8.0)	4 (5.0)	0.66†			
Sweden		**Lumbar spine**			**Left hip (femoral neck)**		
		ACPA-positive	**ACPA-negative**	**p-value**	**ACPA-positive**	**ACPA-negative**	**p-value**
Absolute BMD (g/cm²)	**Baseline EMM (95% CI)**	1.10 (1.06 to 1.14)	1.13 (1.10 to 1.17)	0.12	0.85 (0.83 to 0.88)	0.90 (0.86 to 0.94)	0.22
	Yearly change β (95% CI)	0.003 (-0.002 to 0.009)	0.003 (-0.002 to 0.009)	0.95	-0.003 (-0.007 to 0.001)	-0.003 (-0.009 to 0.002)	0.92
Z-score	**Baseline EMM (95% CI)**	-0.15 (-0.36 to 0.06)	0.02 (-0.21 to 0.26)	0.13	-0.22 (-0.45 to 0.00)	-0.06 (-0.28 to 0.17)	0.12
	Yearly change β (95% CI)	0.035 (0.005 to 0.064)	0.033 (0.002 to 0.064)	0.93	0.004 (-0.026 to 0.034)	0.003 (-0.031 to 0.037)	0.98

	Baseline n (%)	37 (33.0)	16 (20.2)	**0.04**
Osteopenia	**5 years n (%)**	34 (38.6)	25 (39.7)	
	10 years n (%)	26 (44.1)	15 (32.6)	0.56†
	Baseline n (%)	33 (29.5)	26 (32.9)	0.54
Osteoporosis	**5 years n (%)**	28 (31.8)	14 (22.2)	
	10 years n (%)	14 (23.7)	12 (26.0)	0.73†

Table 2. Generalized estimating equations (conducted on 20 imputed datasets) of the effect of ACPA on baseline and longitudinal change in absolute BMD and Z-score and the association between ACPA and the prevalence (%) of osteopenia or osteoporosis over time. The models were adjusted for the following baseline variables: age, sex, BMI, symptom duration and smoking status, as well as the following longitudinal time-varying measurements: DAS44, prednisone intake, HAQ, CRP levels and serum 25-OH vitamin D levels (vitamin D levels only available for the Dutch cohort). Point estimates and 95% confidence intervals are estimated marginal means (EMMs) for baseline BMD and parameter estimates (β) for yearly change BMD. Osteopenia was defined as a -2.5 < T-score ≤ -1.0 at any location and osteoporosis was defined as a T-score≤-2.5 at any location. In the final GEE model, osteopenia was defined as "at least osteopenia", so a T-score≤-1.0 with a T-score >-1.0 as reference. The same was done for osteoporosis, with comparison groups of a T-score≤-2.5 vs a T-score>-2.5. P-values are based on Wald's Chi-squared test of model effects for ACPA (e.g. baseline) and for the ACPA*time interaction (e.g. yearly change). †Yearly change P-value with ACPA-negative as reference group. *P-values that remained significant after correction for multiple testing.

	Lumbar spine				Left hip (total hip)			
	Baseline		Yearly change		Baseline		Yearly change	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Absolute BMD (g/cm²)	-0.002 (-0.017 to 0.126)	0.76	0.001 (-0.001 to 0.002)	0.37	0.007 (-0.006 to 0.019)	0.31	0.0003 (-0.002 to 0.002)	0.80
Z-score	0.001 (-0.129 to 0.132)	0.99	0.004 (-0.009 to 0.017)	0.54	0.074 (-0.016 to 0.165)	0.11	-0.004 (-0.012 to 0.004)	0.33

Table 3. Generalized estimating equations (conducted on non-imputed data) of the association between ACPA IgG levels at inclusion and baseline and longitudinal change in absolute BMD and Z-score. Analyses were performed for Dutch ACPA-positive

	Lumbar spine				Left hip (total hip)			
	Absolute BMD (g/cm²)	**P-value**	**Z-score**	**P-value**	**Absolute BMD (g/cm²)**	**P-value**	**Z-score**	**P-value**
ACPA								
Baseline β (95% CI)	**-0.04 (-0.07 to -0.004)**	**0.03**	**-0.30 (-0.59 to -0.01)**	**0.04**	**-0.03 (-0.06 to -0.01)**	**0.01***	**-0.29 (-0.47 to -0.11)**	**<0.01***
Yearly change β (95% CI)	-0.001 (-0.01 to 0.003)	0.61	-0.01 (-0.04 to 0.02)	0.44	-0.0003 (-0.004 to 0.004)	0.89	0.01 (-0.01 to 0.04)	0.37
ACPA corrected for anti-CarP and RF								
Baseline β (95% CI)	-0.02 (-0.06 to 0.01)	0.18	-0.20 (-0.51 to 0.12)	0.22	**-0.03 (-0.06 to -0.003)**	**0.03**	**-0.28 (-0.48 to -0.07)**	**0.01***
Yearly change β (95% CI)	-0.001 (-0.01 to 0.003)	0.54	-0.01 (-0.05 to 0.02)	0.39	-0.0005 (-0.004 to 0.004)	0.81	0.01 (-0.01 to 0.03)	0.42
RF								
Baseline β (95% CI)	**-0.03 (-0.07 to -0.001)**	**0.04**	-0.27 (-0.57 to 0.04)	0.08	-0.01 (-0.04 to 0.01)	0.27	-0.13 (-0.32 to 0.06)	0.17
Yearly change β (95% CI)	-0.0004 (-0.005 to 0.004)	0.86	-0.02 (-0.05 to 0.01)	0.27	0.0003 (-0.004 to 0.004)	0.88	0.001 (-0.02 to 0.03)	0.93
RF corrected for ACPA								

patients (n=268). Log10-transformation on ACPA IgG levels was applied in order to achieve normal distribution of levels. The models were adjusted for the following baseline variables: age, sex, BMI, symptom duration and smoking status, as well as the following longitudinal time varying measurements: DAS44, prednisone intake, HAQ, CRP levels and serum 25-OH vitamin D levels (vitamin D levels only available for the Dutch cohort). Point estimates and 95% confidence intervals are parameter estimates (β) for baseline and yearly change per 10-fold (or Log10) difference in ACPA IgG levels. P-values are based on Wald's Chi-squared test of model effects for ACPA levels (e.g. baseline) and for ACPA levels*time interaction (e.g. yearly change).

Baseline β (95% CI)	-0.03 (-0.06 to 0.11)	0.18	-0.18 (-0.50 to 0.15)	0.29	-0.001 (-0.03 to -0.03)	0.97	-0.01 (-0.21 to 0.19)	0.93
Yearly change β (95% CI)	0.0001 (-0.004 to 0.005)	0.95	-0.02 (-0.05 to 0.02)	0.41	0.001 (-0.004 to 0.005)	0.77	-0.004 (-0.03 to 0.02)	0.78
Anti-CarP								
Baseline β (95% CI)	-0.02 (-0.05 to 0.01)	0.20	-0.18 (-0.46 to 0.09)	0.19	**-0.03 (-0.05 to -0.001)**	**0.04**	**-0.19 (-0.37 to -0.005)**	**0.04**
Yearly change β (95% CI)	-0.004 (-0.005 to 0.004)	0.85	-0.001 (-0.04 to 0.03)	0.95	0.001 (-0.004 to 0.005)	0.80	0.002 (-0.02 to 0.02)	0.85
Anti-CarP corrected for ACPA								
Baseline β (95% CI)	-0.01 (-0.04 to 0.02)	0.59	-0.08 (-0.37 to 0.21)	0.57	-0.02 (-0.04 to 0.011)	0.24	-0.09 (-0.29 to 0.11)	0.38
Yearly change β (95% CI)	-0.00003 (-0.005 to 0.005)	0.99	0.004 (-0.03 to 0.04)	0.83	0.001 (-0.004 to 0.01)	0.76	-0.002 (-0.03 to 0.02)	0.84
Number of antibodies corrected for ACPA								
Baseline β (95% CI)	-0.01 (-0.04 to 0.01)	0.24	-0.11 (-0.31 to 0.09)	0.63	-0.01 (-0.24 to 0.01)	0.48	-0.06 (-0.19 to 0.07)	0.36
Yearly change β (95% CI)	-0.0004 (-0.002 to 0.002)	0.68	-0.01 (-0.02 to 0.01)	0.37	0.0001 (-0.002 to 0.002)	0.95	0.003 (-0.01 to 0.13)	0.63

Table 4. Generalized estimating equations (conducted on 20 imputed datasets) of the effect of ACPA, RF, anti-CarP and number of antibodies (ACPA, RF, anti-CarP: 0-3) on baseline and longitudinal changes in BMD and Z-score. Data are shown for patients of the Dutch cohort. The models were adjusted for the following baseline variables: age, sex, BMI, symptom duration and smoking status, as well as the following longitudinal time-varying measurements: DAS44, prednisone intake, HAQ, CRP levels and serum 25-OH vitamin D levels (vitamin D levels only available for the Dutch cohort). Point estimates and 95% confidence intervals are parameter estimates (β) for baseline and yearly change. P-values are based on Wald's on Wald's Chi-squared test of model effects for ACPA, RF and anti-CarP (e.g. baseline) and for the antibody*time interaction (e.g. yearly change). *P-values that remained significant after correction for multiple testing.

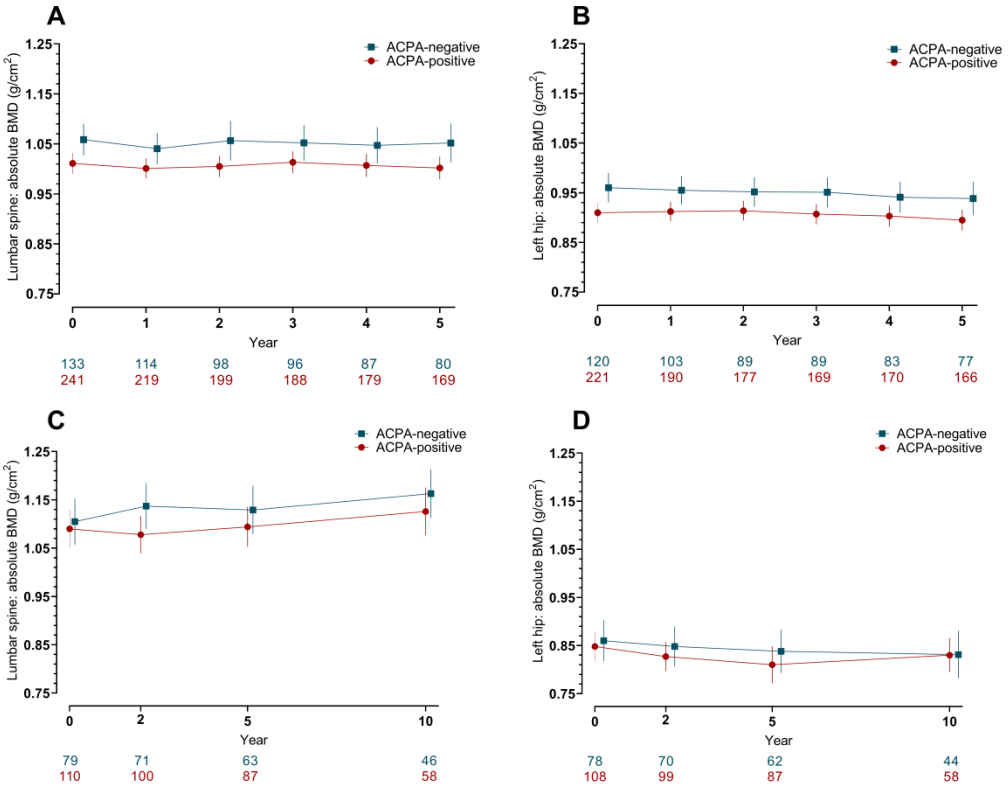
FIGURE LEGENDS

Figure 1. Raw data plots split on ACPA-status, illustrating the yearly change in BMD measurements.

Legend: Graphs A and B are based on data of the Dutch cohort, whereas graphs C and D represent Swedish data. Values below the graphs represent the number of patients with available DXA scores for each given time point in the ACPA-positive and the ACPA-negative group respectively. The error bars show the 95% confidence intervals (CIs) for both groups at the given time points.

Figure 2. Raw data plots split on ACPA-status, illustrating the yearly change in BMD measurements in Dutch patients (n=128) with a mean DAS>1.8 during the first two years after inclusion (baseline visit not included).

Legend: Values below the graphs represent the number of patients with available raw DXA scores for each given time point in the ACPA-positive and the ACPA-negative group respectively. The error bars show the 95% confidence intervals (CIs) for both groups at the given time points.



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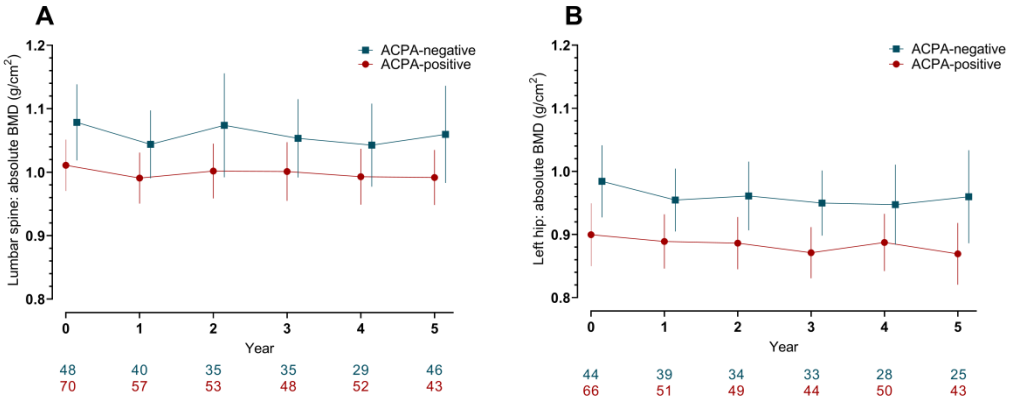

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