osteology

MDPI

Article

The Influence of Anti-Citrullinated Polypeptide Antibodies on Bone Mineral Density Decrease and Incident Major Osteoporotic Fractures in Patients with Rheumatoid Arthritis: A Retrospective Case-Control Study

Ichiro Yoshii [1,*], Tatsumi Chijiwa [2] and Naoya Sawada [3]

[1] Department of Rheumatology and Musculoskeletal Medicine, Yoshii Clinic, M.D. 6-7-5 Nakamura-Ohashidori, Shimanto City 787-0033, Japan
[2] Department of Rheumatology, Kochi Memorial Hospital, Kochi City 780-0824, Japan
[3] Department of Rheumatology, Dohgo Onsen Hospital, Matsuyama 790-0858, Japan
* Correspondence: ichiroyo@giga.ocn.ne.jp; Tel.: +81-880-34-5005; Fax: +81-880-35-5040

Abstract: Background: Effects of anti-citrullinated polypeptide antibodies (ACPA) on the bone mineral density (BMD) reduction and incidence of major osteoporotic fractures (MOF) in patients with rheumatoid arthritis (RA) were evaluated using a retrospective longitudinal case-control study. Methods: Patients with RA who were examined using dual-energy X-ray absorptiometry and simultaneously treated for more than 5 years were recruited. BMD absolute value and Z-scores at initial measurements (baseline) and changes of these values from baseline were assessed, and associations between BMD and candidate risk factors including ACPA positivity and serum titer levels were statistically evaluated. Additional statistical evaluations of ACPA positivity in regard to the incidence of MOF were tested. Results: A total of 222 patients were included. Higher ACPA titers correlated significantly with lower BMD and Z-scores at baseline using a multivariate model ($p < 0.05$). ACPA positivity correlated significantly with lower values and an annual decrease in the Z-score in total hip at follow-up using a univariate model ($p < 0.05$), whereas no significant correlation was found using a multivariate model. Z-scores in the ACPA-positive group were significantly lower than those of the ACPA-negative group ($p < 0.05$). However, ACPA-positivity demonstrated no higher risk for incident MOF. Conclusions: The presence of ACPA is a potential risk of BMD loss however weak.

Keywords: anti-citrullinated polypeptide antibodies; bone mineral density; osteoporotic fracture; rheumatoid arthritis; Z-score

Citation: Yoshii, I.; Chijiwa, T.; Sawada, N. The Influence of Anti-Citrullinated Polypeptide Antibodies on Bone Mineral Density Decrease and Incident Major Osteoporotic Fractures in Patients with Rheumatoid Arthritis: A Retrospective Case-Control Study. *Osteology* **2023**, *3*, 47–60. https://doi.org/10.3390/osteology3020006

Academic Editor: Redha Taiar

Received: 30 December 2022
Revised: 10 March 2023
Accepted: 3 April 2023
Published: 6 April 2023

1. Introduction

It is widely accepted that rheumatoid arthritis (RA) is a determinant risk factor of osteoporosis [1–9]. In the past 10 years, FRAX® (fracture risk assessment tool; a diagnostic tool used to evaluate the 10-year probability of bone fracture risk) has been globally used as an investigative tool for determining the risk of osteoporotic fractures. It includes a questionnaire with items such as glucocorticoid administration, current smoking habits, and bone fragility fracture history of both the patients and their parents [10]. In the questionnaire of FRAX, suffering with RA is included as a risk factor.

RA is associated with a high risk of osteoporotic fracture, and many risk factors of osteoporosis, besides the nature of RA itself, were identified. These are glucocorticoid (GCS) administration [11–13], chronic inflammation [14], impaired mobility due to joint deformity [3,15], sarcopenia (likely to be caused by decreased mobility), polypharmacy, and malnutrition cachexia [16].

As with these risks in RA, the presence of anti-citrullinated polypeptide antibodies (ACPA) is also a critical risk factor for bone loss [17–19]. Loss of the bone matrix was reported in previous animal studies [20]. ACPA differentiates osteoclast precursors, activates osteoclasts, and induces bone resorption. Thus, the presence of ACPA is a potential risk factor for bone loss [21,22]. ACPA may manifest an additional risk for incident fragility fractures [23]. The ACPA titer has also been extracted as one of the risk factors in longitudinal studies of human subjects [24]. However, the risk weight or significance of the proposed ACPA for bone fragility remains unclear. We evaluated these issues using a single-center retrospective case control study.

2. Materials and Methods

We have been treating RA under the treat-to-target strategy (T2T) since August 2010. Diagnoses of RA were undertaken in accordance with the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria of RA [25]. The initial target of therapy was the attainment of remission with a simplified disease activity index (SDAI) within 6 months of initiation [26]. When RA patients were 50 years of age or older or received GCS during treatment, bone mineral density (BMD) in the lumbar spine (LS) and the total hip (TH) in the proximal femur were measured using dual-energy X-ray absorptiometry (DXA) at first consultation or at least within one year since initiation. DXA measurements were made using the DPX® Bravo ME9309 Bone Densitometer (GE Health Care, Chicago, IL, USA: Coefficients of variation; CV: 1.1% (lumbar spine), 0.9% (femoral neck)). These Japanese RA patients were recruited for the study. The baseline was set when the first DXA was measured, and follow-up continued from baseline to 60 months, including the occurrence of the first fragility fracture. Patients who were censored at the time of death or could not be followed were excluded from the study. All patients were followed up in accordance with SDAI and Health Assessment Questionnaire Disability Index (HAQ) monitoring every 1 ~ 3 months. These data were used for analysis. Sharp/van der Heijde Score (SHS) was measured at baseline and annually thereafter, however, we adopted only the data at baseline.

Our primary endpoint was the BMD and Z-score, which mean the number of standard deviations of the BMD for the same gender and generation of the patients in both LS and TH at the closest observation after 5 years of follow-up, and the secondary endpoint was the occurrence of an incident major osteoporotic fracture (MOF) during follow-up.

2.1. Baseline Background Study

We have evaluated a correlation between the BMD in each LS and TH and the background characteristics at baseline. Candidate risk factors were set as independent factors at baseline, such as sex, age, disease duration of RA, positivity (cut off index = 4.5), and serum titer level of ACPA, which were measured using a Clinical Laboratory Improvement Amendments method, rheumatoid factor (RF), SDAI score, HAQ score, PS-VAS, and SHS. In addition to these, the presence of prevalent MOF (pr-MOF), the presence of lifestyle-related diseases (LSD), and cognitive impairment (C-I) were selected as independent factors. Moreover, clearly evident risk factors such as an increased ability to fall or a disorder (fall-ability) was included. MOF included vertebral body fractures, hip fractures, distal radial fractures, proximal humeral fracturess, and other fractures caused by bone fragility. These fractures were identified using both interviews and X-ray pictures. LSD included type 2 diabetes mellitus, chronic obstructive pulmonary disease, hypertension, hyperlipidemia, chronic heart failure, chronic kidney disfunction $\geq$ Stage 3a, and insomnia. Fall-ability included a musculoskeletal ambulation disability complex, osteoarthritis of the lower extremities, joint contractures of the trunk or lower extremities, disuse syndrome, parkinsonism, and neuromuscular disorders. The diagnoses of these comorbidities were made by the authors, who are specialists certified by the Japanese Society of Internal Medicine, the Japanese Orthopaedic Association, and the Japanese College of Rheumatology. We have evaluated

a correlation between the Z-score in LS and TH and candidate risk factors in the same manner as that used to evaluate the BMD at baseline study.

2.2. Follow-Up Study

Correlations between BMD values in each LS and TH at last measure and the candidate risk factors used in BMD at baseline, substituting for the mean values of SDAI and HAQ score during follow-up for these factors at baseline, were also examined. Correlations between the Z-score at the last measure as for BMD and the candidate risk factors were also examined in the same manner.

Correlations between the mean annual change of BMD and Z-score in each LS and TH were also evaluated in the same manner.

2.3. Comparison between ACPA Positive/Negative Groups Study

Furthermore, patients were classified in accordance with ACPA positivity: ACPA-positive (ACPA $\geq$ 4.5U/mL) and ACPA-negative (ACPA < 4.5) groups. Background factors of the two groups were compared at both baseline and follow-up, and the change in the Z-score between the groups was compared for each site of LS and TH.

2.4. Incident MOF Study

We examined the relationship between the occurrence of incident MOF and candidate risk factors during follow-up in the same population using a Cox regression analysis.

We used a linear regression analysis for the baseline background evaluation and for the follow-up evaluations, besides a change of the Z-score analysis, whereas an ANOVA t-test was used for the comparative analysis. For the incident MOF study, we used a binary regression analysis. We identified significant correlated factors within 5% in univariate models and evaluated a multivariate model of these factors for linear regression and binary regression analyses. All statistical analyses were performed using StatPlus:mac Pro® (AnalystSoft, Inc., Walnut, CA, USA).

3. Results

A total of 222 patients were recruited including 17 males (7.7%) and 205 females (92.3%). The mean age of the patients was 69.2 years old. Mean disease duration at baseline and follow-up length after baseline were 6.4 and 63.3 months, respectively. Mean SDAI score, HAQ score, and SHS at baseline were 22.2, 0.516, and 6.6, respectively. The mean ACPA level and positive rate were 202.1 and 77.5%, respectively. Number of patients who already presented MOF at baseline was 115 (51.8%), and incident MOF in the follow-up period was 39 (17.6%) (Table 1).

Table 1. Patients' demographic characteristics at baseline and follow-up.

Cases (Male:Female)	**222 (17:205)**
age at baseline (year-old)	69.2 ± 11.8
disease duration at baseline (months)	6.4 ± 6.8
ACPA positivity (%)	77.5
ACPA titer (U/mL)	202.1 ± 496.4
RF titer at baseline (IU/mL)	95.6 ± 199.8
SDAI at baseline	22.2 ± 8.9
HAQ at baseline	0.516 ± 0.617
SHS at baseline	6.6 ± 7.3
presence of prevalent MOF at baseline	115 (51.8%)
BMD in the LS at baseline and follow-up (g/cm^2)	$0.838 \pm 0.185/0.845 \pm 0.185$
BMD in the TH at baseline and follow-up (g/cm^2)	$0.706 \pm 0.143/0.709 \pm 0.126$
Z-score in the LS at baseline and follow-up	$-0.047 \pm 1.467/0.148 \pm 1.491$
Z-score in the TH at baseline and follow-up	$0.106 \pm 1.061/0.314 \pm 0.949$

Table 1. *Cont.*

Cases (Male:Female)	222 (17:205)
presence of lifestyle-related diseases at baseline (%)	85.5
presence of fall-ability at baseline (%)	66.8
presence of cognitive impairment at baseline (%)	10.7
presence of incident MOF at follow-up	39 (17.6%)
anti-osteoporotic drug administered at baseline and follow-up (%)	45.8 and 66.7
GCS administered at baseline and follow-up (%)	33.8 and 24.6

The values are presented as mean ± SD unless indicated otherwise. In BMD and Z-scores, values at baseline and at follow-up are separated by a slash. Abbreviations: ACPA, anti-citrullinated polypeptide antibodies; RF, rheumatoid factor; SHS, Sharp/van der Heijde Score; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; BMD, bone mineral density; LS, lumbar spine; TH, total hip; GCS, glucocorticoid steroid.

3.1. Baseline Background Study

Female gender, older age, and the presence of pr-MOF significantly correlated negatively with BMD in LS using univariate models, whereas only female gender and the presence of pr-MOF remained in the multivariate model. Female gender, older age, longer disease duration, higher ACPA titer, higher SDAI score, higher HAQ score, higher SHS, and the presence of pr-MOF significantly correlated negatively with BMD in TH using univariate models, and female gender, older age, longer disease duration, higher ACPA titer, and the presence of pr-MOF also correlated using a multivariate model (Table 2).

Female gender, ACPA positivity, and the presence of fall-ability significantly correlated with the Z-score in LS using univariate models, and the presence of fall-ability was the only factor that correlated with the Z-score in LS positively at baseline using a multivariate model. Longer disease duration, ACPA positivity, higher ACPA titer, higher HAQ score, higher SHS, the presence of pr-MOF, and the presence of fall-ability significantly correlated with the Z-score in TH using univariate models, and longer disease duration, higher ACPA titer, and the presence of fall-ability correlated using a multivariate model (Table 2).

3.2. Follow-Up Study

The BMD in LS at last observation significantly correlated negatively with female gender and the presence of pr-MOF using univariate models. Both variables also correlated using a multivariate model. The BMD in TH significantly correlated negatively with female gender, older age, longer disease duration, higher mean SDAI score, higher mean HAQ score, higher SHS at baseline, and the presence of pr-MOF using univariate models. Female gender, older age, and the presence of pr-MOF significantly correlated using a multivariate model (Table 3).

Table 2. Correlation of candidate variables with BMD and Z-scores at baseline.

Candidate Risk Factors	BMD in LS		BMD in TH		Z-Score in LS		Z-Score in TH	
	Univariate Models *p*-Value	Multivariate Model (R: 0.413) Coefficient (95%CI) (Beta-Value)	Univariate Models *p*-Value	Multivariate Model (R: 0.572) Coefficient (95%CI) (Beta-Value)	Univariate Models *p*-Value	Multivariate Model (R: 0.211) Coefficient (95%CI) (Beta-Value)	Univariate Models *p*-Value	Multivariate Model (R: 0.440) Coefficient (95%CI) (Beta-Value)
female	**<0.001**	**−0.248 (−0.332–−0.164) (−0.358) ###**	**<0.001**	**−0.078 (−0.145–−0.010) (−0.143) #**	**<0.01**	−0.319 (−1.086–0.448) (−0.059)	0.19	
older age	**<0.05**	−0.001 (−0.003–−0.001) (−0.080)	**<0.001**	**−0.004 (−0.005–−0.010) (−0.299) ###**	0.1		0.13	
longer disease duration	0.86		**<0.05**	**−0.005 (−0.008–−0.017) (−0.234) ##**	0.8		**<0.001**	**−0.035 (−0.063–−0.008) (−0.220) #**
ACPA positivity	0.31		0.61		**<0.05**	−0.343 (−0.7632–0.047) (−0.125)	**<0.05**	−0.026 (−0.344–0.293) (−0.012)
higher ACPA titer	0.54		**<0.05**	**−0.000 (−0.000–−0.000) (−0.140) #**	0.41		**<0.01**	**−0.000 (−0.001–−0.000) (−0.153) #**
higher SDAI score	0.16		**<0.05**	0.000 (−0.002–0.162) (0.011)	0.36		0.11	
higher HAQ score	0.89		**<0.01**	−0.001 (−0.033–0.030) (−0.006)	0.37		**<0.05**	−0.069 (−0.305–0.167) (−0.042)
higher SHS	0.22		**<0.001**	−0.000 (−0.001–0.000) (0.118)	0.29		**<0.001**	−0.002 (−0.004–0.001) (−0.123)
pr−MOF	**<0.001**	**−0.071 (−0.120–−0.023) (−0.193) ##**	**<0.001**	**−0.055 (−0.094–−0.017) (−0.202) ##**	0.1		**<0.05**	−0.254 (−0.551–0.043) (−0.122)
LSDs	0.42		0.16		0.51		0.15	
Fall	0.20		0.95		**<0.01**	**0.432 (0.027–0.838) (0.153) #**	**<0.05**	**0.352 (0.044–0.660) (0.162) #**
CI	0.44		0.07		0.67		0.78	
OPD administration	0.83		0.89		0.4		0.41	
GCS administration	0.27		0.43		0.44		0.9	

Statistical procedure: linear regression analysis. Bold font represents significance within 5%. #, <0.05; ##,<0.01; ###, <0.001. Abbreviations: BMD, bone mineral density; MOF, major osteoporotic fracture; LS, lumbar spine; TH, total hip; ACPA, anti-citrullinated polypeptide antibodies; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; SHS, Sharp/van der Heijde Score; pr-MOF, presence of prevalent major osteoporotic fractures; LSDs, presence of lifestyle-related diseases; Fall, presence of hyper fall-ability; CI, presence of cognitive impairment; OPD, anti-osteoporotic drugs; GCS, glucocorticoid steroid.

Table 3. Correlation of candidate risk factors with BMD and Z-scores during follow-up.

Being Female	**<0.001**	**−0.217 (−0.305–−0.131) (−0.314) ###**	**<0.001**	**−0.100 (−0.154–−0.045) (−0.216) ###**	**<0.001**	**−0.831 (−1.556–−0.106) (−0.152) #**	**0.16**	
older age at last contact	0.11		**<0.001**	**−0.003 (−0.004–−0.001) −0.261 ###**	0.3		0.19	
longer disease duration at last contact	0.91		**<0.001**	−0.002 (−0.005–0.000) −0.129	0.99		**<0.01**	**−0.029 (−0.056–0.003) (−0.188) #**
ACPA positivity at baseline	0.2		0.82		**<0.05**	−0.377 (−0.774–0.020) (−0.134)	**<0.05**	−0.121 (−0.391–0.149) (−0.066)
higher ACPA titer at baseline	0.34		0.47		0.71		0.14	
higher mean SDAI score at follow-up	0.14		**<0.01**	−0.003 (−0.007–0.001) (−0.110)	0.07		**<0.001**	−0.028 (−0.062–0.005) (−0.127)
higher mean HAQ score at follow-up	0.98		**<0.001**	−0.016 (−0.047–0.014) (−0.076)	0.46		0.08	
higher mean SHS at follow-up	0.45		**<0.001**	−0.000 (−0.000–0.000) (−0.099)	0.20		**<0.001**	−0.001 (−0.003–0.001) (−0.071)
pr-MOF at baseline	**<0.01**	**−0.055 (−0.101–−0.008) (−0.148) #**	**<0.001**	**−0.048 (−0.081–−0.016) (−0.197) ###**	0.28		0.1	
LSDs, ever	0.65		0.32		0.35		0.51	
Fall, ever	0.31		0.94		**<0.05**	0.089 (−0.479–0.302) (0.031)	**<0.05**	0.110 (−0.366–0.146) (−0.006)
CI, ever	0.52		0.29		0.95		0.75	
OPD administration, ever	0.86		0.92		0.45		0.63	
GCS administration, ever	0.39		0.64		0.59		0.67	

Statistical procedure: linear regression analysis. Bold font represents significance within 5%. #, <0.05; ###, <0.001. Abbreviations: BMD, bone mineral density; MOF, major osteoporotic fracture; LS, lumbar spine; TH, total hip; ACPA, anti-citrullinated polypeptide antibodies; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; SHS, Sharp/van der Heijde Score; pr-MOF, presence of prevalent major osteoporotic fractures; LSDs, presence of lifestyle-related diseases; Fall, presence of hyper fall-ability; CI, presence of cognitive impairment; OPD, anti-osteoporotic drug; GCS, glucocorticoid steroid.

A higher Z-score in LS significantly correlated with female gender, ACPA positivity, and the presence of fall-ability using univariate models, and female gender was the only factor that correlated with the Z-score using a multivariate model. A higher Z-score in TH significantly correlated negatively with longer disease duration, ACPA positivity, higher mean SDAI score, and higher mean SHS, and the presence of fall-ability significantly correlated positively with Z-score using univariate models. However, using a multivariate model, longer disease duration was the only significant factor (Table 3).

The annual Increase in BMD in LS significantly corelated with female gender and the presence of pr-MOF, positively, and with GCS administration, negatively, using univariate models. Among these, no candidate factors correlated significantly using a multivariate model. The annual increase in BMD in TH significantly correlated only with higher SHS using univariate models, and it also correlated with the annual increase in BMD in TH using a multivariate model (Table 4).

The annual increase in Z-score in LS significantly correlated with female gender, ACPA positivity, and the presence of fall-ability using univariate models, and only the ACPA positivity significantly correlated with the annual increase in Z-score using a multivariate model. The annual increase in Z-score in TH significantly correlated with longer disease duration, the ACPA positivity, higher mean SDAI score, higher SHS, and the presence of fall-ability during follow-up using univariate models. Among these, higher mean SDAI score was the only factor that correlated significantly with the annual increase in Z-score in TH using a multivariate model (Table 4).

3.3. Comparison for ACPA Positivity Study

The mean SDAI score, RF, and SHS at baseline in the ACPA-positive group were significantly higher than those in the ACPA-negative group, whereas the mean age and Z-score in LS in the ACPA-positive group was significantly lower than those in the ACPA-negative group. During follow-up, SHS in the ACPA-positive group was significantly higher than that in the ACPA-negative group, whereas Z-score in LS and the mean annual increase in Z-score in both LS and TH were significantly lower in the ACPA-positive group than those in the ACPA-negative group (Table 5). The other parameters showed no significant difference between the two groups.

3.4. Incident MOF Study

Older age, higher HAQ score, higher SHS, higher BMD in LS and TH, the presence of LSD, fall-ability, and CI had significantly higher risk ratios for pr-MOF at baseline using univariate models, whereas older age, higher SHS, the presence of fall-ability, and CI showed significant higher risk ratios even using a multivariate model.

Higher HAQ score at baseline, higher mean HAQ score at follow-up, the presence of pr-MOF at baseline, the presence of LSD, and fall-ability had significantly higher risk ratios for incident MOF, whereas the presence of pr-MOF was the only factor that showed a significantly higher risk ratio for incident MOF using a multivariate model, and neither ACPA positivity nor higher ACPA titer showed significantly higher risk ratios (Table 6).

Table 4. Correlation of candidate risk factors with the annual increase in BMD and Z-score.

Candidate Risk Factors	BMD in LS (120/102)		BMD in TH (121/101)		Z-Score in LS		Z-Score in TH	
	Univariate Models	Multivariate Model (R: 0.227)	Univariate Models	Multivariate Model (R: 0.332)	Univariate Models	Multivariate Model (R: 0.188)	Univariate Models	Multivariate Model (R: 0.418)
	p-Value	Coefficient (95%CI) (Beta−Value)	*p*-Value	Coefficient (95%CI) (Beta-Value)	*p*-Value	Coefficient (95%CI) (Beta-Value)	*p*-Value	Coefficient (95%CI) (Beta-Value)
being female	**<0.001**	0.128 (−0.372–0.628) (0.035)	0.32		**<0.001**	0.111 (−0.451–0.675) (−0.028)	0.16	
older age at last contact	0.27		0.92		0.3		0.19	
longer disease duration at last contact	0.60		0.08		0.99		**<0.01**	−0.008 (−0.037–0.022) (−0.047)
ACPA positivity at baseline	0.76		0.91		**<0.05**	**−0.384 (−0.668–0.099) (0.075) ##**	**<0.05**	−0.032 (−0.333–0.268) (−0.016)
higher ACPA titer at baseline	0.51		0.53		0.71		0.14	
higher mean SDAI score at follow-up	0.96		0.57		0.06		**<0.001**	**−0.044 (−0.081–−0.007) (−0.186) #**
higher mean HAQ score at follow-up	0.62		0.42		0.46		0.07	
higher mean SHS at follow-up	0.61		**<0.05**	**0.003 (0001–0.005) (0.177) #**	20		**<0.001**	−0.001 (−0.003–0.002) (−0.052)
pr-MOF at baseline	**<0.05**	0.136 (−0.135–0.47) (0.068)	0.14		0.28		0.1	
LSDs, ever	0.22		0.99		0.35		0.51	
Fall, ever	0.29		0.69		**<0.05**	0.156 (−0.141–0.453) (0.075)	**<0.05**	0.092 (−0.207–0.391) (0.045)
CI, ever	0.98		0.61		0.95		0.75	
OPD administration, ever	0.85		0.29		0.45		0.63	
GCS administration, ever	**<0.05**	−0.221 (−0.509–0.067) (−0.105)	0.2		0.59		0.67	

Statistical procedure: linear regression analysis. Bold font represents significance within 5%. #, <0.05; ##, <0.01; Abbreviations: BMD, bone mineral density; MOF, major osteoporotic fracture; LS, lumbar spine; TH, total hip; ACPA, anti-citrullinated polypeptide antibodies; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; SHS, Sharp/van der Heijde Score; pr-MOF, presence of prevalent major osteoporotic fractures; LSDs, presence of lifestyle-related diseases; Fall, presence of hyper fall-ability; CI, presence of cognitive impairment; OPD, anti-osteoporotic drug; GCS, glucocorticoid steroid.

Table 5. Comparison between the two groups.

	Parameters	ACPA-Positive (n = 172)	ACPA-Negative (n = 50)	p-Value
at baseline	female (%)	91.3	96.5	0.10
	age (year-old)	65.4	71.3	**<0.001**
	disease duration (months)	7.7	4.6	**<0.001**
	RF (IU/L)	138.3 ± 197.1	21.5 ± 49.3	**<0.001**
	SDAI	26.3 ± 24.0	21.0 ± 17.8	**<0.05**
	HAQ	0.496 ± 0.618	0.553 ± 0.639	0.48
	SHS	8.4 ± 8.2	3.5 ± 5.0	**<0.001**
	BMD in LS (g/cm^2)	0.825 ± 0.167	0.849 ± 0.156	0.23
	BMD in H (g/cm^2)	0.700 ± 0.140	0.710 ± 0.132	0.75
	Z-score in LS	−0.246 ± 1.300	0.123 ± 1.392	**<0.05**
	Z-score in TH	−0.062 ± 1.034	0.261 ± 1.020	**<0.05**
	presence of lifestyle-related disease (%)	87.5	85.5	0.69
	presence of fall-ability (%)	69.8	64.2	0.49
	presence of cognitive impairment (%)	9.8	10.8	0.82
at follow-up	follow-up length (months)	64.8	65.4	0.65
	SDAI	4.5 ± 3.1	5.1±4.4	0.22
	HAQ	0.495 ± 0.616	0.516 ± 0.544	0.32
	SHS	8.1 ± 8.2	3.4 ± 4.8	**<0.001**
	BMD in LS (g/cm^2)	0.839 ± 0.171	0.870 ± 0.165	0.16
	BMD in TH (g/cm^2)	0.710 ± 0.118	0.713 ± 0.115	0.99
	Z-score in LS	−0.008 ± 1.361	0.368 ± 1.426	**<0.05**
	Z-score in TH	0.129 ± 0.902	0.396 ± 0.891	0.11
	anti-osteoporotic drug administered, ever (%)	73.4	69.8	0.72
	GCS administered, ever (%)	35.8	32.9	0.68

The values are presented as mean ± SD unless indicated otherwise. Abbreviations: RF, rheumatoid factor; SHS, Sharp/van der Heijde Score; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; BMD, bone mineral density; LS, lumbar spine; TH, total hip; GCS, glucocorticoid steroid. Statistically significant within 0.05 is shown in bold font.

Table 6. Correlation of candidate risk factors for BMD and prevalent MOF occurrence at baseline.

	Prevalent MOF		Incident MOF	
	Univariate Model	Multivariate Model	Univariate Model	Multivariate Model
	Odds Ratio (95%CI)	Odds Ratio (95%CI) (Beta-Value)	Odds Ratio (95%CI)	Odds Ratio (95%CI) (Beta-Value)
female gender	1.59 (0.58–4.34)		3.64 (0.47–28.30)	
older age at baseline	**1.08 (1.05–1.11) ###**	**1.08 (1.04–1.12) (0.08) ###**	1.00 (0.97–1.03)	
longer disease duration at baseline	1.03 (0.99–1.08)		1.01 (0.96–1.06)	
ACPA positivity at baseline	1.6 (0.66–2.03)		0.84 (0.41–1.73)	
higher ACPA titer at baseline	1.00 (1.00–1.00)		1.00 (1.00–1.00)	
higher SDAI score at baseline	1.02 (0.99–1.06)		1.03 (1.00–1.08)	
Higher mean SDAI score at follow-up			1.04 (0.97–1.11)	
Higher HAQ score at baseline	**2.24 (1.35–3.70) ##**	1.05 (0.56–1.98) (0.05)	**1.72 (1.02–2.91) #**	1.21 (0.38–3.82) (0.19)
Higher mean HAQ score at follow-up			**1.75 (1.03–2.96) #**	2.7 (0.32–3.66) (0.07)
higher SHS at baseline	**1.00 (1.00–1.01) #**	**1.01 (1.00–1.01) (0.01) #**	1.00 (1.00–1.01)	

Table 6. *Cont.*

	Prevalent MOF		Incident MOF	
	Univariate Model	**Multivariate Model**	**Univariate Model**	**Multivariate Model**
	Odds Ratio (95%CI)	**Odds Ratio (95%CI) (Beta-Value)**	**Odds Ratio (95%CI)**	**Odds Ratio (95%CI) (Beta-Value)**
presence of prevalent MOF at baseline			**6.77 (2.71–16.95) ###**	**4.85 (1.84–12.79) (1.58) ##**
higher BMD in the LS at baseline	**0.05 (0.01–0.27) ###**	0.16 (0.01–2.41) (−1.82)	0.50 (0.07–3.48)	
higher BMD in the LS at last observation			1.08 (0.17–6.95)	
higher BMD in the TH at baseline	**0.01 (0.00–0.02) ###**	0.04 (0.00–1.73) (−3.35)	0.13 (0.01–1.61)	
higher BMD in the TH at last observation			0.18 (0.01–3.13)	
presence of LSDs, ever	**2.83 (1.28–6.29) #**	2.22 (0.78–6.32) (0.80)	**8.39 (1.11–63.36) #**	5.01 (0.62–40.37) (1.61)
presence of fall-ability, ever	**3.48 (1.91–6.32) ###**	**2.88 (1.34–6.19) (1.06) ##**	**3.35 (1.33–8.42) #**	1.84 (0.67–5.05) (0.61)
presence of cognitive impairment, ever	**12.02 (2.74–52.67) ###**	**6.63 (1.19–37.05) (1.89) #**	1.71 (0.63–4.67)	
anti-osteoporotic drug administration, ever	0.73 (0.41–1.30)		0.63 (0.31–1.29)	
GCS administration, ever	0.86 (0.49–1.51)		0.65 (0.30–1.43)	

Statistical procedure: BMD, linear regression analysis; prevalent MOF, binary logistic regression analysis. Bold font represents significance within 5% (#, <0.05; ##,<0.01; ###, <0.001). Abbreviations: BMD, bone mineral density; MOF, major osteoporotic fracture; LS, lumbar spine; TH, total hip; ACPA, anti-citrullinated polypeptide antibodies; SDAI, simplified disease activity index; HAQ, Health Assessment Questionnaire Disability Index; SHS, Sharp/van der Heijde Score; LSDs, lifestyle-related diseases; GCS, glucocorticoid steroid.

4. Discussion

It may be clear from previous reports that the production of ACPA triggers the development of RA and joint and bone destruction mechanisms [27–29]. It also predictably causes bone fragility. ACPA stimulates osteoclast differentiation and activation in animal or in vitro studies [17,18]. ACPA has been reported to be involved in bone resorption through the induction of osteoclasts from the synovial membrane before the onset of RA [17]. Since the main locus of ACPA involvement in bone resorption is the synovium, the cortical bone at the joint periphery is considered to be the main site of bone resorption. Its involvement in systemic bone resorption in clinical practice has only been reported in cross-sectional studies [20,21], and there are few reports that describe the role of ACPA based on a longitudinal cohort study, to the best of our knowledge [24,27]. This cohort study assessed the extent to which ACPA contributes to bone fragility using retrospective longitudinal case-control data from a single center in clinical practice.

In selecting candidate risk factors, commonly established risk factors such as female gender, older age, and BMD were included. Recently focused risk factors for osteoporotic fracture, namely, presenting diabetes mellitus, chronic obstructive pulmonary diseases, chronic kidney disfunction, and insomnia were included together in the presence of LSD. In addition to these, hyper fall-ability was included because we expected fracture risk to increase proportionally with an increase in fall-ability.

Disease-activity level was represented with the SDAI score, daily-life activity level was represented with the HAQ score, the duration of suffering RA was represented with disease duration, and joint deformity level was represented with SHS, which were also included as RA-specific factors. Drug factors such as anti-osteoporotic drug and glucocorticoid were also included. However, the family history of MOF could not be included because the

patients' memories were vague and unreliable as all subjects were elderly. Preferential habits, such as smoking and drinking alcohol, were also excluded because there were very few subjects exhibiting these in the study.

The reason for adopting the Z score over the T score is that the T score represents the relative deviation from the mean BMD within the 30 s of each gender, but not in the same generation as the participants. Previous reports indicated that ACPA was not a determinant of BMD, such as as gender and age [20–22]. Due to the need to exclude effects of gender and age, Z scores were adopted, but T scores were not included in the study. The reasons for not selecting subjects younger than 50 years were the same.

The results showed that commonly established risk factors, such as female gender, older age, and the presence of pr-MOF, had a greater impact on BMD at both LS and TH, particularly on BMD at TH, which was affected by more factors than BMD at LS.

We conclude that ACPA has an effect, but not a strong effect, on bone mineral density. In the baseline study, higher ACPA titers were significantly associated with a lower BMD and Z-score in TH, but ACPA titers were the weakest of five significant risk factors followed by older age, longer disease duration, presence of pr-MOF, and female gender. There was no significant correlation in the univariate model of LS. However, ACPA positivity was significantly associated with lower Z-scores in both LS and TH, and higher ACPA titer levels were significantly associated with lower Z-scores in TH. In the follow-up study, ACPA was not a significant risk factor for the absolute BMD value, but ACPA positivity was associated with lower Z-scores in TH using a univariate model; however, no significant correlation showed using a multivariate model. The effect of ACPA on BMD was evidently shown in its annual changes. There showed no significant correlation between the annual change in BMD and both ACPA positivity and titer, however, ACPA positivity showed a significant correlation with the annual change in Z-score for both LS and TH using a univariate model, while no significant correlation was shown using a multivariate model.

From these results, ACPA may contribute to total body BMD loss, and its effect is probably independent. In particular, one significant marker of this was ACPA positivity and not higher ACPA titers. This suggests that the relative decrease in bone mineral density is independent of the antibody titer of ACPA and, more importantly, depends on the presence of ACPA itself. The ACPA is tested by measuring serum levels and is likely to differ from bone level or synovial fluid level. Therefore, the positive ACPA level reflects the accelerated bone resorption in the bone. However, it is weak among various risk factors, and its effects are offset by multivariate models. Furthermore, the absence of any correlation between ACPA positivity and changes in absolute BMD may indicate that ACPA has a weak effect on systemic bone resorption. Thus, ACPA positivity affected baseline BMD and Z-score, but not follow-up BMD. In other words, other factors such as control of disease activity, GCS administration, and changes in patient motility may be confounding.

There are substantial non-ACPA-positive confounders, including age, sex, body mass index, the duration of disease, disease activity, activities of daily living, joint deformity level, joint contracture, etc. However, the results of the controlled trial indicate that the distribution of women in the ACPA-positive group is significantly lower than those in the ACPA-negative group, and the mean age of the ACPA-positive group is significantly younger, which suppresses BMD reduction at baseline. Among other factors, the SDAI score and SHS at baseline were significantly higher in the ACPA-positive group than in the ACPA-negative group, but the SDAI score at follow-up showed no significant difference between the two groups. Nor did the HAQ score at both baseline and follow-up show a significant difference. These results indicate that ACPA positivity is an independent factor in decreasing relative BMD in the clinic; in other words, ACPA-positive patients are at risk for decreasing BMD compared with ACPA-negative patients.

However, ACPA positivity does not contribute to the development of MOF. In the incident MOF study, results showed that the presence of prevalent MOF was the only factor among the candidate factors, which included ACPA positivity and higher titer of ACPA. A lower BMD was not a significant factor. This suggests that lower BMD does not always

mean a higher risk for incident MOF, but past fracture history is a more important factor for the occurrence of incident MOF.

Anti-osteoporotic drugs had no effect on BMD or Z-score, and GCS was similarly ineffective. Although the bias of the subject pool, which is the dataset of the present study, may be a problem, the effect of these drugs may not be strong enough to offset the individual difference of the subjects. Also, the possibility that the sample size of 222 was too small for proving a significant effect cannot be denied.

Biological and targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs) had no effect on BMD or Z-score, although they may affect ACPA titers. Rituximab, which is thought to have the greatest effect on ACPA titers, was not covered by insurance for RA in Japan and was not administered to study subjects. Therefore, b/tsDMARD is unlikely to affect BMD or Z-score through ACPA titer in this study population.

There were several limitations in the study. First, this was a single-center retrospective study, therefore, the number of subjects was rather small, and there is a risk of patient selection and ethnicity bias. Another is that biological disease-modifying anti-rheumatic drug administration during follow-up was not considered. Furthermore, this was a longitudinal study, but ACPA itself included only baseline values as its source of information, despite the fact that the ACPA titer may change during follow-up. However, ACPA antibody titers were unlikely to change with treatment, and it is difficult to treat changes during treatment as independent risk factors. Our conclusion is that serum ACPA-positive individuals have a relative risk of bone fragility, but not a strong risk, which remains unchanged in the presence of limitation.

Author Contributions: Conceptualization, I.Y.; Methodology, I.Y. and N.S.; Software, I.Y.; Validation, I.Y., T.C. and N.S.; Formal Analysis, I.Y.; Investigation, I.Y.; Resources, I.Y., T.C. and N.S.; Data Curation, I.Y.; Writing—Original Draft Preparation, I.Y.; Writing—Review & Editing, I.Y., T.C. and N.S.; Visualization, I.Y.; Supervision, T.C. and N.S.; Project Administration, I.Y.; Funding Acquisition, none. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Yoshii Hospital's Ethics Committee (approval number: Y-OP-2020-3, approved on 14 November 2020).

Informed Consent Statement: Anonymity was ensured for all patients and families who participated in this study, and no names and/or addresses were issued that could help identify these individuals.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: Authors would like to thank Kaoru Kuwabara, Sayori Masuoka, Eri Morichika, and Aoi Yoshida for their dedicated data collection. The authors would also like to thank Enago (www.enago.jp) for the enthusiastic English language review.

Conflicts of Interest: None of authors or their families have shares, income, property with any person, or any grants or other financial support that present a conflict of interest with the study.

Abbreviations

ACPA, anti-citrullinated polypeptide antibodies; RF, rheumatoid factor; BMD, bone mineral density; RA, rheumatoid arthritis; DXA, dual-energy X-ray absorptiometry; TH, total hip; LS, lumbar spine; FRAX, fracture assessment tool; GCS, glucocorticoid steroid; T2T, treat-to-target strategy; SDAI, simplified disease activity index; FN, femoral neck; GT, greater trochanter; PS-VAS, pain score using visual analog scale; HAQ, Health Assessment Questionnaire Disability Index; SHS, Sharp/van der Heijde Score; MOF, major osteoporotic fracture; pr-MOF, prevalent major osteoporotic fracture; LSD, lifestyle-related diseases; Fall-ability, increased ability to fall or a disorder; CI, cognitive impairment; b/tsDMARD, biological and targeted synthetic disease-modifying anti-rheumatic drug.

References

1. Hooyman, J.R.; Melton, L.J., 3rd; Nelson, A.M.; O'Fallon, W.M.; Riggs, B.L. Fractures after rheumatoid arthritis. A population-based study. *Arthritis Rheum.* **1984**, *27*, 1353–1361. [CrossRef] [PubMed]
2. Hall, G.M.; Spector, T.D.; Griffin, A.J.; Jawad, A.S.; Hall, M.L.; Doyle, D.V. The effect of rheumatoid arthritis and steroid therapy on bone density in postmenopausal women. *Arthritis Rheum.* **1993**, *36*, 1510–1516. [CrossRef] [PubMed]
3. Haugeberg, G.; Uhlig, T.; Falch, J.A.; Halse, J.I.; Kvien, T.K. Bone mineral density and frequency of osteoporosis in female patients with rheumatoid arthritis: Results from 394 patients in the Oslo County Rheumatoid Arthritis register. *Arthritis Rheum.* **2000**, *43*, 522–530. [CrossRef] [PubMed]
4. Huusko, T.M.; Korpela, M.; Karppi, P.; Avikainen, V.; Kautiainen, H.; Sulkava, R. Threefold increased risk of hip fractures with rheumatoid arthritis in Central Finland. *Ann. Rheum. Dis.* **2001**, *60*, 521–522. [CrossRef]
5. Lodder, M.C.; de Jong, Z.; Kostense, P.J.; Molenaar, E.T.; Staal, K.; Voskuyl, A.E.; Hazes, J.M.W.; Dijkmans, B.A.C.; Lems, W.F. Bone mineral density in patients with rheumatoid arthritis: Relation between disease severity and low bone mineral density. *Ann. Rheum. Dis.* **2004**, *63*, 1576–1580. [CrossRef]
6. Van Staa, T.P.; Geusens, P.; Bijlsma, J.W.; Leufkens, H.G.; Cooper, C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis Rheum.* **2006**, *54*, 3104–3112. [CrossRef]
7. Güler-Yüksel, M.; Allaart, C.F.; Goekoop-Ruiterman, Y.P.; de Vries-Bouwstra, J.K.; van Groenendael, J.H.; Mallée, C.; De Bois, M.H.W.; Breedveld, F.C.; Dijkmans, B.A.C.; Lems, W.F. Changes in hand and generalised bone mineral density in patients with recent-onset rheumatoid arthritis. *Ann. Rheum. Dis.* **2009**, *68*, 330–336. [CrossRef]
8. Kim, S.Y.; Schneeweiss, S.; Liu, J.; Daniel, G.W.; Chang, C.L.; Garneau, K.; Solomon, D.H. Risk of osteoporotic fracture in a large population-based cohort of patients with rheumatoid arthritis. *Arthritis Res. Ther.* **2009**, *12*, R154. [CrossRef]
9. Heidari, B.; Roushan, M.R.H. Rheumatoid arthritis and osteoporosis. *Caspian J. Intern. Med.* **2012**, *3*, 445–446.
10. Kanis, J.A. FRAX® Fracture Risk Assessment Tool. University of Sheffield. 2011. Available online: https://www.sheffield.ac.uk/FRAX/tool.aspx?lang=en/ (accessed on 27 April 2021).
11. Cortet, B.; Flipo, R.M.; Blanckaert, F.; Duquesnoy, B.; Marchandise, X.; Declamble, B. Evaluation of bone mineral density in patients with rheumatoid arthritis. Influence of disease activity and glucocorticoid therapy. *Rev. Rheum. Engl. Ed.* **1997**, *64*, 451–458.
12. Van Staa, T.P.; Leufkens, H.G.M.; Abenhaim, L.; Zhang, B.; Cooper, C. Use of oral corticosteroid and risk of fractures. *J. Bone Miner. Res.* **2000**, *15*, 993–1000. [CrossRef]
13. Kanis, J.A.; Johansson, H.; Oden, A.; Johnell, O.; de Laet, C.; Melton, L.J., III; Tenenhouse, A.; Reeve, J.; Silman, A.J.; Pols, H.A.; et al. A meta-analysis of prior corticosteroid use and fractur risk. *J. Bone Miner. Res.* **2004**, *19*, 893–899. [CrossRef]
14. Cortet, B.; Guyot, M.H.; Solau, E.; Pigny, P.; Dumoulin, F.; Flipo, R.M.; Marchandise, X.; Delcambre, B. Factors influencing bone loss in rheumatoid arthritis: A longitudinal study. *Clin. Exp. Rheumatol.* **2000**, *18*, 683–690.
15. Furuya, T.; Inoue, E.; Hosoi, T.; Taniguchi, A.; Momohara, S.; Yamanaka, H. Risk factors associated with the occurrence of hip fracture in Japanese patients with rheumatoid arthritis: A prospective observational cohort study. *Osteoporos. Int.* **2013**, *24*, 1257–1265. [CrossRef]
16. Gosch, M.; Jeske, M.; Kammerlander, C.; Roth, T. Osteoporosis and polypharmacy. *J. Gerontol. Geriat.* **2012**, *45*, 450–454. [CrossRef]
17. Krishnamurthy, A.; Joshua, V.; Haj Hensvold, A.; Jin, T.; Sun, M.; Vivar, N.; Ytterberg, A.J.; Engström, M.; Fernandes-Cerqueira, C.; Amara, K.; et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Ann. Rheum. Dis.* **2016**, *75*, 721–729. [CrossRef]
18. Steffen, U.; Schett, G.; Bozec, A. How Autoantibodies Regulate Osteoclast Induced Bone Loss in Rheumatoid Arthritis. *Front. Immunol.* **2019**, *10*, 1483. [CrossRef]
19. Krishnamurthy, A.; Ytterberg, A.J.; Sun, M.; Sakuraba, K.; Steen, J.; Joshua, V.; Tarasova, N.K.; Malmström, V.; Wähämaa, H.; Réthi, B.; et al. Citrullination Controls Dendritic Cell Transdifferentiation into Osteoclasts. *J. Immunol.* **2019**, *202*, 3143–3150. [CrossRef]
20. Catrina, A.; Krishnamurthy, A.; Rethi, B. Curent view on the pathogenic role of anti-citrullinated protein antibodies in rheumatoid arthritis. *RMD Open* **2021**, *7*, e001228. [CrossRef]
21. Kleyer, A.; Finzel, S.; Rech, J.; Manger, B.; Krieter, M.; Faustini, F.; Araujo, E.; Hueber, A.J.; Harre, U.; Engelke, K.; et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann. Rheum. Dis.* **2014**, *73*, 854–860. [CrossRef]
22. Llorente, I.; Merino, L.; Ortiz, A.M.; Escolano, E.; González-Ortega, S.; García-Vicuña, R.; García-Vadillo, J.A.; Castañeda, S.; González-Álvaro, I. Anti-citrullinated protein antibodies are associated with decreased bone mineral density: Baseline data from a register of early arthritis patients. *Rheumatol. Int.* **2017**, *37*, 799–806. [CrossRef] [PubMed]
23. Stemmler, F.; Simon, D.; Liphardt, A.M.; Englbrecht, M.; Rech, J.; Hueber, A.J.; Engelke, K.; Schett, G.; Kleyer, A. Biomechanical properties of bone are impaired in patients with ACPA-positive rheumatoid arthritis and associated with the occurrence of fractures. *Ann. Rheum. Dis.* **2018**, *77*, 973–980. [CrossRef] [PubMed]
24. Yoshii, I.; Sawada, N.; Chijiwa, T.; Kokei, S. Impact of sustaining SDAI remission for preventing incident of bone fragility fracture with rheumatoid arthritis. *Ann. Rheum. Dis.* **2022**, *81*, 296–299. [CrossRef] [PubMed]

25. Aletaha, D.; Neogi, T.; Silman, A.J.; Funovits, J.; Felson, D.T.; Bingham, C.O., 3rd; Birnbaum, N.S.; Burmester, G.R.; Bykerk, V.P.; Cohen, M.D.; et al. 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* **2010**, *62*, 2569–2581. [CrossRef]
26. Smolen, J.S.; Landewé, R.B.M.; Bijlsma, J.W.J.; Burmester, G.R.; Dougados, M.; Kerschbaumer, A.; Mclnnes, I.B.; Sepriano, A.; Vollenhoven, R.F.; Wit, M.; et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann. Rheum. Dis.* **2020**, *79*, 685–699. [CrossRef]
27. Wu, C.Y.; Yang, H.Y.; Lai, J.H. Anti-citrullinated protein antibodies in patients with rheumatoid arthritis: Biological effects and mechanisms of immunopathogenesis. *Int. J. Mol. Sci.* **2020**, *21*, 4015. [CrossRef]
28. Sokolova, M.V.; Schett, G.; Steffen, U. Autoantibodies in rheumatoid arthritis: Histological background and novel findings. *Clin. Rev. Allergy Immunol.* **2022**, *63*, 138–151. [CrossRef]
29. Amkreutz, J.A.; de Moel, E.C.; Theander, L.; Willim, M.; Heimans, L.; Nilsson, J.Å.; Karlsson, M.K.; Huizinga, T.W.; Åkesson, K.E.; Jacobsson, L.T.; et al. Association Between Bone Mineral Density and Autoantibodies in Patients with Rheumatoid Arthritis. *Arthritis Rheumatol.* **2021**, *73*, 921–930. [CrossRef]