

Association Between Serum Bone Biomarker Levels and Therapeutic Response to Abatacept in Patients With Rheumatoid Arthritis (Ra): a Multicenter Ra Ultrasound Prospective Cohort Study in Japan

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Abstract

Background: To evaluate the effect of treatment on serum bone biomarkers and explore whether serum bone biomarkers are associated with therapeutic response in rheumatoid arthritis (RA) patients treated with abatacept.

Methods: We enrolled 59 RA patients treated with abatacept from a multicenter prospective ultrasound cohort study of patients who received biologic or targeted synthetic disease-modifying antirheumatic drug (DMARD) therapy. We evaluated the patients' clinical disease activity and musculoskeletal ultrasound (MSUS) scores. The serum concentrations of five bone biomarkers were evaluated (Dickkopf-1 [Dkk-1], sclerostin [SOST], osteocalcin [OC], osteopontin [OPN], and osteoprotegerin [OPG]) by multiplex bead assays at baseline, 3, and 6 months: the change over 6 months was defined as the Δ value. 'Power Doppler (PD) responder' was defined as a patient whose Δ total PD score over 6 months was greater than the median change.

Results: Abatacept significantly improved clinical disease activity as well as the MSUS score over 6 months. Serum OPG was significantly elevated at 6 months after the introduction of abatacept ($p=0.016$). The Δ SOST and Δ OPG values were negatively correlated with the Δ total PD score ($r_s = -0.31, p < 0.05$ and $r_s = -0.34, p < 0.01$, respectively). The serum Dkk-1 at baseline was significantly lower in the PD responders ($n=30$) compared to the non-PD responders ($n=29$) ($p=0.026$). A multivariate logistic regression analysis showed that the serum Dkk-1 at baseline (odds ratio 0.50, 95% confidence interval [CI] 0.23–0.91, $p=0.043$) was an independent predictor of PD responder status.

Conclusion: Serum levels of bone biomarkers may be useful for predicting RA patients' therapeutic response to abatacept.

Name of the registry: Assessment of therapeutic responsiveness by imaging of the joints in patients with rheumatoid arthritis; A observational cohort study

Trial registration number: UMIN000012524

Date of registration: 12/9/2013

URL of trial registry record: https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000014657

Introduction

Rheumatoid arthritis (RA) is characterized by persistent synovitis, systemic inflammation, and autoantibodies [1]. Uncontrolled active RA causes joint damage, disability, decreased quality of life, and comorbidities. The tight control of the disease activity of RA following the treat-to-target (T2T) strategy is thus recommended [2]. Advances in the treatment of RA, such as biological disease-modifying anti-rheumatic drugs (bDMARDs), have provided better clinical outcomes, including the achievement of

clinical remission, the prevention of joint damage, and the preservation of function for individuals with RA.

Abatacept, a fusion protein of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and immunoglobulin G1, selectively modulates the CD80/CD86:CD28 costimulatory signal required for full T-cell activation [3]. Abatacept is an effective treatment for patients with RA, according to both clinical trials [4–7] and practice [8]. Abatacept is thus recommended as one of the first-line bDMARDs for RA [9]. Several studies showed that abatacept strongly inhibits radiographic progression in patients with RA [5–8]. CTLA-4 is suggested to be an anti-osteoclastogenic molecule that directly binds osteoclast precursor cells and inhibits their differentiation [10, 11].

Bone damage in RA is characterized by articular erosions, periarticular bone loss, and secondary osteoporosis caused by chronic inflammation [12]. In the bone metabolism of RA osteoclastogenesis/bone resorption is promoted and bone formation is inhibited. Among the underlying mechanisms, important roles are played by the interaction of immune responses mediated by proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)-6 and IL-17, and bone biomarkers such as receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (Dkk-1), and sclerostin (SOST) [12–15]. Effects of bDMARDs including TNF inhibitors [16, 17] and IL-6 inhibitors [18–20] on bone biomarkers in patients with RA have been reported, but the effect of abatacept on bone biomarkers in patients with RA has not been investigated. Here, we measured the serum levels of five bone biomarkers (Dkk-1, SOST, osteocalcin [OC], osteopontin [OPN], and OPG) using a multiplex bead assay (bone panel) in RA patients treated with abatacept.

Dkk1 and SOST inhibit the Wnt signaling pathway regulating bone formation [12, 14, 15]. OC is an important component of bone matrix protein, synthesized mainly in osteoblasts [12, 21], and it is a biomarker of bone formation. OPG is a soluble decoy RANKL receptor that inhibits RANKL function, and it is highly involved in inflammatory bone resorption by interfering with RANK–RANKL binding [12, 14, 15]. OPN is strongly expressed in bone, where it promotes the adhesion of osteoclasts to the mineralized matrix regulating bone resorption and formation [22]. OPN has also been recognized as an important proinflammatory mediator which participates in pathological processes such as inflammatory reactions, vasospasm formation, and bone damage [23, 24].

To achieve the goal of the T2T strategy, the adequate management of disease activity requires a sensitive and accurate assessment of arthritis. Imaging plays an important role in this assessment. Musculoskeletal ultrasound (MSUS) has been widely applied in clinical settings as an imaging modality for patients with rheumatic diseases [25, 26]. Compared to clinical and radiographic examinations, MSUS provides a straightforward and more accurate detection of both inflammation and damage at the joint level [25, 26]. We have conducted a multicenter prospective observational cohort study of patients with active RA who received bDMARDs or targeted synthetic DMARDs (tsDMARDs) therapy at 27 participating rheumatology centers from the Kyushu region of Japan since June 2013 [Kyushu Multicenter Rheumatoid Arthritis Ultrasound Prospective Observational Cohort Study (KUDOS)] [27–29]. We evaluated the

therapeutic efficacy of the bDMARDs and tsDMARDs by clinical measurements, MSUS, and biomarker assessments. A multicenter collaborative study that prospectively evaluates disease activity by using MSUS standardized at a high level is rare, even worldwide.

In the present study, we evaluated the effect of abatacept treatment on bone biomarkers and explored whether bone biomarkers are associated with the therapeutic response in RA patients treated with abatacept using the KUDOS.

Materials And Methods

Patients

This study is part of an ongoing non-randomized multicenter prospective cohort study [KUDOS] of patients with active RA who received bDMARD or tsDMARD therapy at 27 participating rheumatology centers from the Kyushu region of Japan since June 2013 [27–29]. We evaluated therapeutic efficacy by determining the patients' clinical disease activity, MSUS score, and serum biomarkers at baseline and at 3, 6, 9, 12, 18, and 24 months starting from the initiation of new bDMARD or tsDMARD. For the present study, we enrolled the 59 consecutive Japanese patients with active RA who were treated with abatacept and had continued the treatment for > 6 months at 10 participating rheumatology centers during the period from December 2013 to March 2016. All patients were required to satisfy the 1987 American College of Rheumatology [30] and/or 2010 American College of Rheumatology (ACR)/EULAR criteria for RA [31]. Abatacept was administered as recommended by the manufacturers: 125 mg via subcutaneous injection weekly or 500–750 mg via intravenous infusion every 4 weeks. We excluded patients who have newly introduced an oral bisphosphonate during the study period or treated with intravenous bisphosphonates, anti-RANKL antibodies, or parathyroid hormone (PTH) agents.

The study is registered with the University Hospital Medical Information Network Clinical Trials Registry (<http://www.umin.ac.jp/ctr/>, UMIN 000012524) and was approved by the Institutional Review Board of Nagasaki University (approval no. 13102866). All patients gave their signed informed consent to participate in accordance with the Helsinki Declaration.

Clinical and laboratory assessments

Disease activity was evaluated by each of the attending physicians (Japan College of Rheumatology [JCR]-certified rheumatologists) according to the Disease Activity Score (DAS) 28- C-reactive protein (CRP) value and Simple Disease Activity Index (SDAI) value at baseline and every 3 months after the introduction of abatacept. The treating physicians were different from the MSUS evaluators. The baseline MSUS scores were evaluated after the decision regarding the introduction of b/tsDMARDs therapy.

Musculoskeletal ultrasound assessment

The MSUS examination of each patient was performed by JCR-certified sonographers. At all of the participating institutions, a trained MSUS expert examined the patient in a situation recommended by the

JCR guidelines, paying attention to factors that can affect PD results, including the room temperature, the last use of a nonsteroidal anti-inflammatory drug (NSAID), and hand position. Medium-level to high-level ultrasound machines were used (Toshiba AplioXG and Aplio300, GE Logic series 7 and 8 or Hitachi Ascendus, Avius, Noblus, and Hi Vision Preirus) with high-frequency (12–18.5 MHz) linear transducers. The Doppler parameters were adjusted according to the device used (range of pulse repetition frequency 500–1000 Hz; Doppler frequency 6.1–10.0 MHz). There was no change in MSUS settings during the study.

Twenty-two joints including the metacarpophalangeal (MCP), proximal interphalangeal (PIP), and wrist joints of the bilateral hands were assessed by MSUS at baseline and at 3 and 6 months of treatment. The 22 joints were scanned on the dorsal aspect. Standardized joint and probe positions were used, based on a guideline published by the JCR. Each grayscale (GS) synovial hypertrophy and PD signal was scored semi-quantitatively on a scale from 0 to 3 [32]. The sum of the GS or PD scores was used as the indicator of US disease activity, described as the total GS score or total PD score. The total scores ranged from 0 to 66. We defined a PD responder at 6 months as a patient whose change in total PD score (Δ total PD score) over 6 months was greater than the median change (i.e., a Δ total PD score less than or equal to -4) in all patients. We defined PD remission as a total PD score of 0 at 6 months. Interobserver reliability was confirmed in a previous investigation [27].

Bone biomarker measurements

We measured the concentrations of the following biomarkers by using serum stored on the same day as the patient's clinical evaluation. Rheumatoid factor (RF) was measured by the latex agglutination turbidimetric immunoassay (LATIA) (LZ test 'Eiken' RF). Anti-cyclic citrullinated peptide antibody (ACPA) was measured by a chemiluminescent immunoassay (CLEIA) (STACIA MEBLux test CCP). We performed the multiplex bead assays using diluted serum supernatants and a Milliplex MAP Human Bone Panel analyzed with a Bio-Plex® MAGPIX™ Multiplex Reader (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. The bone biomarkers that could be measured by the bead panel included Dkk-1, SOST, OC, OPN, and OPG using a multi-suspension array. These bone biomarkers were also measured in 18 healthy age- and gender-matched volunteers (median age 70 years, 67% females). The volunteers were recruited at medical check-ups in the town of Saza, Japan. The protocol was approved by the Nagasaki University Ethics Committee for Humans Subjects (approval no. 14051404).

Statistical analyses

Statistical analyses were performed using JMP Pro statistical software, ver. 11.0 (SAS, Cary, NC). Quantitative variables are presented as medians and interquartile ranges (IQRs). Categorical variables are presented as percentages. We used the Mann-Whitney U-test for comparisons between independent medians, and we used the Chi-square test for the evaluation of the associations between categorical variables. Correlations were assessed with Spearman's correlation coefficient. The changes in clinical disease activity indices, MSUS scores, or serum concentrations of bone biomarkers over 6 months were analyzed using the Wilcoxon signed ranks test. We attempted to identify any variables that were

independently predictive of the PD responder status at 6 months from the patients' baseline characteristics by performing a multivariate logistic regression analysis. All variables with a p-value < 0.1 in a univariate analysis were used in the multivariate models, but the total GS score was excluded as a confounding factor of the total PD score. P-values < 0.05 were considered significant.

Results

Demographic, clinical, and laboratory characteristics of the 59 RA patients

The patient's characteristics at baseline are summarized in Table 1. The median (IQR) age of the patients was 72 (65–77) years, and the median (IQR) of the RA disease duration was 54 (14–186) months. The median (IQR) of the DAS28-CRP and SDAI were 4.20 (3.39–5.01) and 20.7 (13.7–30.7), respectively. The median (IQR) of the total GS and PD scores were 14 (7–22) and 7 (4–15), respectively. Methotrexate (MTX, median dose: 8 mg weekly) and low-dose oral glucocorticoids (median dose: 5 mg daily) were concomitant in 26 (44.1%) and 33 (55.9%) patients, respectively. Twenty-two (37.3%) patients had a history of previous use of bDMARDs.

Table 1
Demographic, clinical and laboratory characteristics of the 59 patients with RA

Age, yrs	72 (65–77)
Male, n	16 (27.1)
Disease duration, months	54 (14–186)
csDMARDs use, n	48 (81.4)
MTX use, n	26 (44.1)
Corticosteroid use, n	33 (55.9)
Previous use of bDMARDs	22 (37.3)
Oral bisphosphonate use	21 (35.6)
Positive RF, n	46 (78.0)
Positive ACPA, n	52 (88.1)
Tender joint counts (28), n	5 (3–10)
Swollen joint counts (28), n	5 (2–10)
PtGA, mm	40 (20–70)
EGA, mm	40 (30–53)
CRP, mg/dl	0.64 (0.12–2.14)
MMP-3, ng/ml	124 (66–288)
DAS28-CRP	4.20 (3.39–5.01)
SDAI	20.7 (13.7–30.7)
Total GS score	14 (7–22)
Total PD score	7 (4–15)
The data are median (interquartile range, $Q_1 - Q_4$) or number (percentage). ACPA: anti-cyclic citrullinated peptide antibody, bDMARDs: biologic disease-modifying antirheumatic drugs, CRP: C-reactive protein, DAS: Disease Activity Score, csDMARDs: conventional synthetic disease-modifying antirheumatic drugs, EGA: evaluator's global assessment, ESR: erythrocyte sedimentation rate, GS: gray-scale, MTX: methotrexate, PD: power Doppler, PtGA: patient's global assessment, RA: rheumatoid arthritis, RF: rheumatoid factor, SDAI: Simplified Disease Activity Index.	

Improvement of clinical and MSUS activities over 6 months

Overall, the patients' DAS28-CRP (Fig. 1A) and SDAI (Fig. 1B) values were significantly improved at 3 and 6 months compared to the baseline ($p < 0.001$, respectively). The median (IQR) of the total GS scores decreased from 14 (7–22) at baseline to 9 (5–18) at 3 months ($p < 0.05$) and 9 (4–17) at 6 months ($p <$

0.05) (Fig. 1C). In addition, the median (IQR) of the PD scores decreased from 7 (4–15) at baseline to 4 (1–11) at 3 months ($p < 0.05$) and 2 (0–7) at 6 months ($p < 0.001$) (Fig. 1D).

The serum concentrations of the bone biomarkers

The results of our comparison of the serum concentrations of the five bone biomarkers between the healthy volunteers and the RA patients and the changes of the biomarkers over the 6-month period after the introduction of abatacept in the RA patients are shown in Supplementary Table S1. Serum OPN was significantly higher ($p < 0.0001$) and serum OC tended to be lower ($p = 0.099$) in the RA patients compared to the healthy volunteers. The other serum bone biomarkers were not significantly different between the RA and healthy groups. Serum OPG was significantly elevated at 6 months after the introduction of abatacept ($p = 0.016$, Suppl. Fig. S1), but the other serum bone biomarkers did not change after treatment. The oral corticosteroids and bisphosphonates did not affect the serum concentrations of bone biomarkers or their changes.

The correlations between the bone biomarkers and disease activity

Table 2 shows the correlations between the serum levels of the bone biomarkers and the disease activity at baseline and the changes (Δ value) over the 6 months of abatacept treatment. At baseline, the serum level of Dkk-1 was positively correlated with the CRP level ($r_s = 0.276$, $p < 0.05$), and that of OC tended to be negatively correlated with the CRP level ($r_s = -0.254$, $p < 0.1$). The serum level of OPG was positively correlated with the SDAI value ($r_s = 0.264$, $p < 0.05$) and total GS score ($r_s = 0.276$, $p < 0.05$), and it tended to be positively correlated with the total PD score ($r_s = 0.232$, $p < 0.1$).

Table 2
Correlations between serum bone biomarkers and disease activity

Baseline					
	Dkk-1	SOST	OC	OPG	OPN
CRP	0.276**	-0.137	-0.254*	0.057	0.173
MMP-3	0.116	0.064	-0.177	0.199	0.220*
DAS28-CRP	0.078	-0.088	-0.004	0.178	0.119
SDAI	0.054	-0.049	0.015	0.264**	0.146
Total GS score	0.057	0.006	0.056	0.276**	0.154
Total PD score	0.027	-0.107	-0.042	0.232*	0.096
Δvalue (change over 6 months)					
	ΔDkk-1	ΔSOST	ΔOC	ΔOPG	ΔOPN
ΔCRP	0.240	-0.264**	-0.161	-0.263**	0.057
ΔMMP-3	0.237*	-0.098	-0.041	-0.034	0.113
ΔDAS28-CRP	-0.027	-0.260**	-0.177	-0.309**	-0.122
ΔSDAI	0.018	-0.191	-0.167	-0.256*	-0.151
ΔTotal GS score	-0.144	-0.223*	-0.030	-0.228*	-0.080
ΔTotal PD score	-0.194	-0.314**	-0.167	-0.342***	-0.127
The results were obtained using Spearman's correlation coefficient. *p < 0.1, **p < 0.05, ***p < 0.01. OC: osteocalcin, OPG: osteoprotegrin, OPN: osteopontin, SOST: sclerostin.					

The ΔSOST was negatively correlated with the ΔCRP ($r_s = -0.264$, $p < 0.05$), the ΔDAS28-CRP ($r_s = -0.260$, $p < 0.05$), and the Δ total PD score ($r_s = -0.314$, $p < 0.05$), and it tended to be negatively correlated with the Δtotal GS score ($r_s = -0.223$, $p < 0.1$). The ΔOPG was negatively correlated with the ΔCRP ($r_s = -0.263$, $p < 0.05$), the ΔDAS28-CRP ($r_s = -0.309$, $p < 0.05$), and the Δtotal PD score ($r_s = -0.342$, $p < 0.01$), and it tended to be negatively correlated with the ΔSDAI ($r_s = -0.256$, $p < 0.1$) and the Δtotal GS score ($r_s = -0.228$, $p < 0.1$).

The association between the ultrasonographic response and the bone biomarkers

Because the ΔSOST and ΔOPG were negatively correlated with the Δtotal PD score as described above, we compared these two parameters between the PD responders and non-PD responders. Both the ΔSOST and ΔOPG were significantly greater in the PD responders compared to the non-PD responders ($p = 0.0041$ and 0.0073 , respectively, Fig. 2).

The results of our comparison of the patient characteristics at baseline between the PD responders and non-PD responders at 6 months are provided in Table 3. The patient age and disease duration tended to be higher in the PD responders compared to the non-PD responders. RF positivity ($p = 0.030$), the SDAI ($p = 0.0081$), the total GS score ($p = 0.0005$), and the total PD score ($p < 0.0001$) were significantly higher in the PD responders compared to the non-PD responders. The serum level of Dkk-1 was significantly lower in the PD responders compared to the non-PD responders ($p = 0.026$). The serum level of SOST tended to be significantly lower in the PD responders compared to the non-PD responders ($p = 0.058$).

Table 3
Comparison of patient characteristics between the PD responders and non-PD responders

	PD responders n = 30	Non-PD responders n = 29	p-value
Age, yrs	74 (67–78)	70 (62–74)	0.058
Male, n	8 (26.7)	8 (27.6)	1.00
Disease duration, months	92 (26–258)	39 (10–156)	0.071
Positive RF, n	27 (90.0)	19 (65.5)	0.030
Positive ACPA, n	28 (93.3)	24 (82.8)	0.21
SDAI	25.0 (16.6–36.5)	18.2 (12.2–24.5)	0.0081
Total GS score	19 (13–28)	8 (4–15)	0.0005
Total PD score	11 (7–20)	4 (1–7)	< 0.0001
Dkk-1, pg/ml	3040 (2489–3652)	3699 (2676–4477)	0.026
SOST, pg/ml	2428 (1721–3057)	2803 (2137–3916)	0.058
OC, pg/ml	16588 (9582–28327)	17722 (12512–39103)	0.54
OPG, pg/ml	958 (749–1190)	945 (839–1534)	0.32
OPN, pg/ml	23035 (17382–40435)	22928 (17305–35962)	0.93
ACPA: anti-cyclic citrullinated peptide antibody, OC: osteocalcin, OPG: osteoprotegrin, OPN: osteopontin, PD: power Doppler, RF: rheumatoid factor, SDAI: Simplified Disease Activity Index, SOST: sclerostin.			

Based on the results of the univariate analysis, we entered six baseline variables (age, disease duration, RF positivity, total PD score, serum Dkk-1 level, and serum SOST level) into the multivariate regressions (Table 4). The serum level of Dkk-1 at baseline (odds ratio [OR] 0.497, 95% confidence interval [CI] 0.225–0.909, $p = 0.043$) was revealed as the only independent predictor of PD responder status at 6 months.

Table 4
Baseline predictors of PD responder at 6 months by multivariate logistic regression analysis

	Comparison	OR	95%CI	p-value
Serum Dkk-1	1 ng/ml increase	0.497	0.225–0.909	0.043
Serum SOST	1 ng/ml increase	0.562	0.286–1.001	0.065
Total PD score	1 increase	1.145	0.989–1.372	0.098
RF	positive	3.751	0.684–29.05	0.154
Disease duration	1 year increase	1.004	0.996–1.011	0.343
Age	1 year increase	1.009	0.934–1.092	0.811
PD: power Doppler, RF: rheumatoid factor, SOST: sclerostin.				

We compared the changes in the SDAI and the total PD score between the patients with a low Dkk-1 value (i.e., < the median Dkk-1 value at baseline) and the patients with a high Dkk-1 value (\geq the median Dkk-1 at baseline) (Suppl. Fig. S2) and between the patients with a low SOST value (< the median SOST at baseline) and those with a high SOST value (\geq the median of SOST at baseline) (Suppl. Fig. S3). The SDAI score improved in the patients with a low Dkk-1 as well as the patients with a high Dkk-1 and in the patients with a low SOST as well as the patients with a high SOST. However, the improvement in the total PD score was better in the patients with a low Dkk-1 compared to those with a high Dkk-1.

Discussion

We evaluated the association between the serum concentrations of five bone biomarkers and the therapeutic response to abatacept in RA patients, using our multicenter prospective ultrasound cohort study [KUDOS]. In the present study, since abatacept significantly improved the patients' clinical disease activity as well as their MSUS score over the 6-month treatment, bone destruction was expected to be prevented in this population.

First, we observed that both Dkk-1 and SOST (which are inhibitors of the Wnt signaling pathway) were associated with the therapeutic response, especially the ultrasonographic response evaluated by the total PD score. Low serum levels of Dkk-1 at baseline was an independent predictor of the PD responder status at 6 months. Low serum levels of SOST at baseline tended to be associated with the PD responder status at 6 months. Increased serum levels of SOST were significantly correlated with the improvement of disease activity after treatment with abatacept. The Wnt signaling pathway plays a key role in several biological processes such as cellular proliferation and tissue regeneration, and its dysregulation is involved in the pathogenesis of many autoimmune diseases [14]. In RA, Wnt signaling is implicated in systemic and localized bone loss. This process involves proinflammatory cytokines produced by the synovial membrane, which may increase bone resorption but also stimulate soluble antagonists of the

canonical Wnt/ β -catenin signaling pathway (including DKK-1 and sclerostin) and subsequently inhibit osteoblast proliferation, maturation, and progenitor differentiation [14, 33, 34].

In particular, the pro-inflammatory cytokines TNF and IL-1 induce Dkk-1 and SOST; IL-17 down-regulates the Wnt/ β -catenin pathway indirectly, enhancing the production of TNF and IL-1; and IL-6 induces the differentiation of B cells into plasma cells which express Dkk-1 [14, 35, 36]. Moreover, Dkk-1 induces SOST [14]. It has been demonstrated that Dkk-1 promotes synovial angiogenesis, a critical process in the pathogenesis of RA [34]: vascular proliferation occurs during pannus formation in the affected joints [37, 38], during which the synovium becomes locally invasive at the interface with cartilage and bone. The serum level of Dkk-1 was shown to be higher in patients with RA than in controls and to correlate with bone erosions and inflammation [34]. Increased serum levels of Dkk-1 and SOST may therefore indicate a poor prognosis and resistance to treatment in patients with RA. In the present patient series, the serum levels of Dkk-1 and SOST at baseline were closely associated with the responsiveness of PD activity, which reflects synovial angiogenesis [37, 39] and predicts joint destruction [40].

Regarding the effect of bDMARD therapy on the Wnt signaling in RA, most of the relevant studies were conducted with TNF α inhibitors [34, 41] or an IL-6 receptor (IL-6R) inhibitor, tocilizumab [18, 19], and they showed a decrease in the serum level of Dkk-1 in RA patients undergoing these bDMARD treatments. A reduction in the serum level of Dkk-1 may result from the inhibition of the TNF α - and IL-6-dependent induction of Dkk-1 by TNF α inhibitors or anti-IL-6 monoclonal antibody. On the other hand, abatacept did not reduce the serum level of Dkk-1 in the present RA patients, perhaps because it does not directly inhibit TNF α and IL-6. There is no report regarding the effect of abatacept, except the present study, on the Wnt signaling in RA patients [14].

In healthy mice, abatacept promoted bone formation by inducing the Wnt ligand Wnt-10b in a T cell-dependent manner [42], but paradoxically it increased the expression of SOST [41]. Bone formation has been suggested to be moderated by a direct negative feedback loop involving a putative CTLA-4 Ig association with CD80/CD86 on the osteoblasts, causing the production of sclerostin [14, 43]. In the present patient series, an increased serum level of SOST was significantly correlated with the improvement of disease activity after the introduction of abatacept.

We also observed that the serum level of OPG was significantly elevated after the introduction of abatacept, and this elevation was significantly correlated with the improvement of disease activity. The RANK-RANKL system is the major driver of bone destruction in inflammatory arthritis [12, 14, 15]. This system is promoted by proinflammatory cytokines such as TNF α and IL-6 [12, 14, 15]. OPG, a decoy receptor of RANKL, influences bone erosions in RA [15, 44]. A low OPG/RANKL ratio has thus been associated with increased radiographic damage in RA [15, 45]. Wnt signaling is involved in osteoclastogenesis regulation; the canonical Wnt/ β -catenin signaling pathway leads to an up-regulation of OPG and a down-regulation of RANKL [14, 35]. TNF inhibitors [14, 46] or IL-6R inhibitor [14, 18, 19] increased both the expression of OPG and the OPG/RANKL ratio, possibly due to a promotion of Wnt signaling following a decrease in DKK-1. Similarly, abatacept may elevate the serum level of OPG

because it promotes Wnt signaling as described above. In addition, the serum levels of OPN among our patients were higher and those of OC tended to be lower compared to the healthy volunteers as in previous reports [21, 23]. However, we did not observe any effect of abatacept treatment on these bone biomarkers.

Some limitations of our study should be mentioned. The limited sample size (n = 59) does not allow for subanalyses of differences due to the patients' heterogeneous characteristics. However, our results are valuable as a part of a multicenter collaborative study that prospectively and closely evaluates disease activity using MSUS. Second, we could not evaluate structural changes in joints. In the cohort study, we evaluated the patients' X-ray images at baseline, 6, 12, 18, and 24 months. We will explore whether bone biomarkers are associated with radiographic progression in RA patients treated with abatacept.

Conclusions

This is the first study to evaluate the effects of abatacept treatment on bone biomarkers in RA patients and to explore whether bone biomarkers are associated with the patients' therapeutic response using the KUDOS. Abatacept may prevent bone destruction through the promotion of the Wnt signaling pathway. In addition, the measurements of the serum levels of bone biomarkers may be useful for predicting the ultrasonographic response to abatacept.

Abbreviations

ACPA
anti-cyclic citrullinated peptide antibody, ACR:American College of Rheumatology, bDMARD:biologic disease-modifying antirheumatic drug, CRP:C-reactive protein, CTLA-4:cytotoxic T lymphocyte-associated antigen-4, DAS:Disease Activity Score, Dkk-1:Dickkopf-1, DMARD:disease-modifying antirheumatic drug, EULAR:European League Against Rheumatism, GS:grayscale, IQR:interquartile range, JCR:Japan College of Rheumatology, KUDOS:Kyushu Multicenter Rheumatoid Arthritis Ultrasound Prospective Observational Cohort Study, MCP:metacarpophalangeal, MSUS:musculoskeletal ultrasound, OC:osteocalcin, OPG:osteoprotegerin, OPN:osteopontin, PD:power Doppler, PIP:proximal interphalangeal, RA:rheumatoid arthritis, SDAI:Simple Disease Activity Index, SOST:sclerostin, T2T:treat-to-target, tsDMARD:targeted synthetic DMARD

Declarations

Ethics approval and consent to participate:

The study was approved by the Institutional Review Board of Nagasaki University (Approval No. 13102866). All patients gave signed informed consent.

Consent for publication: Not applicable

Availability of data and materials:

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests:

AK received research funding from Ono Pharmaceutical Co., Sanofi K.K., Asahi Kasei Pharma Corp., Taisho Toyama Pharmaceutical Co., Teijin Pharma Co., Sumitomo Dainippon Pharma Co., Ltd., Chugai Pharmaceutical Co., MSD Co., Kowa Pharmaceutical Co. Ltd., Taisho Toyama Pharmaceutical Co., Pfizer Japan Inc., Kyowa Hakko Kirin Co., Boehringer Ingelheim Japan, Abbvie GK, Astellas Pharma Inc., Eli Lilly Japan, Daiichi Sankyo Co., Takeda Pharmaceutical Co., Kissei Pharmaceutical Co., Mitsubishi Tanabe Pharma Co., Otsuka Pharmaceutical Co., Cosmic Corporation Co. Ltd., Nihon Medi-Physics Co., Ltd., Daiichi Sankyo Co., Bristol-Myers Squibb, Janssen Pharmaceutical K.K., Mochida Pharmaceutical Co., Ltd., AstraZeneca Co., YL Biologics Co., Alexion GK, and Eisai Co. The other authors declare that they have no competing interests.

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Authors' contributions:

Conceptualization and Methodology: SK, AK. Formal analysis: SK, YE. Investigation: SK, YE, AN, MO, ST, AT, TS, RS, TK, NI, KI, HN, TA, YU, TY, NE, NM, AO, KF, HH, SN, YT. Data Curation: YE, AN. Writing - Original Draft: SK. Writing - Review & Editing; TO, AK. Supervision and Funding acquisition : AK. All authors read and approved the final manuscript.

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Figures

Figure 1

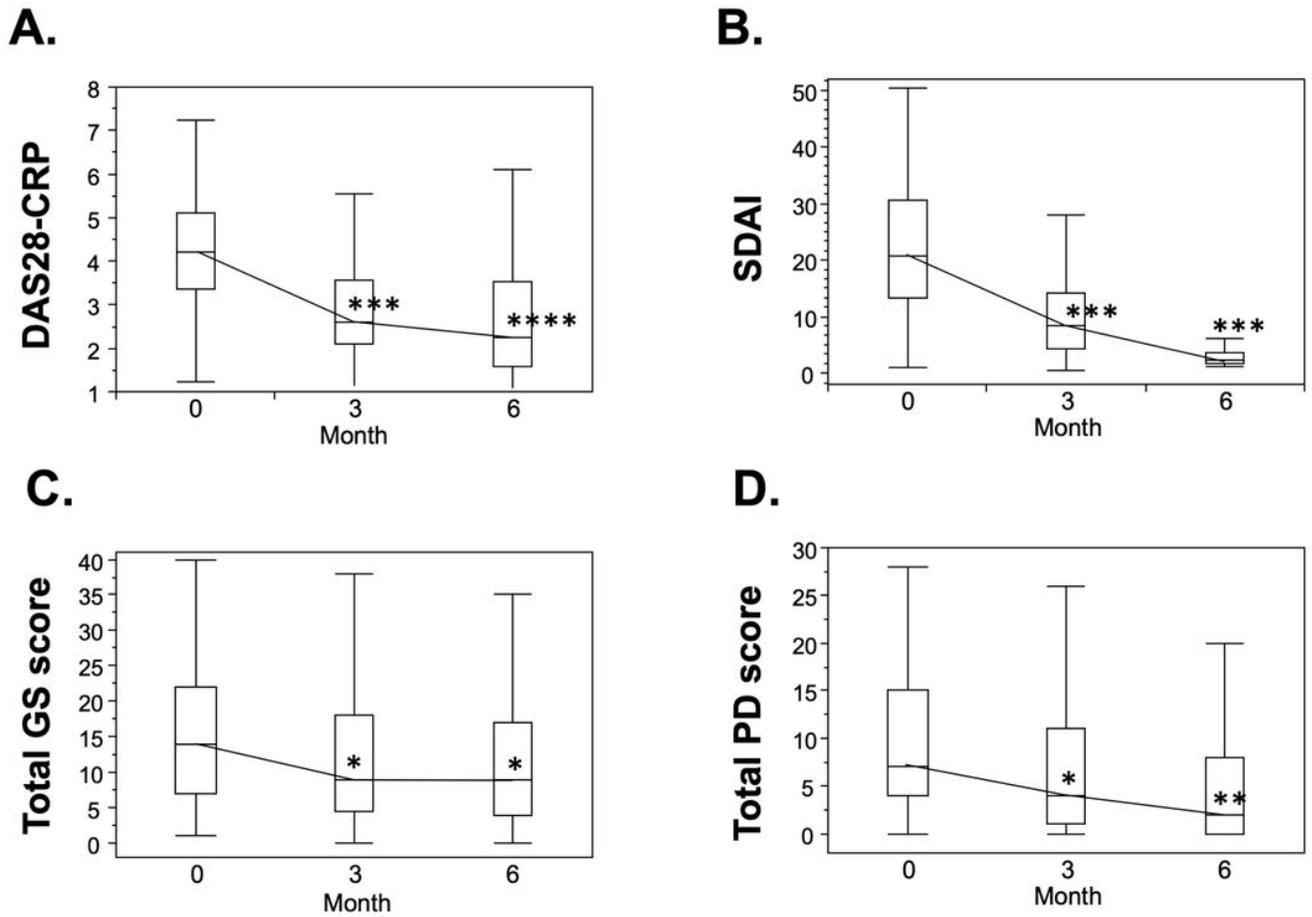


Figure 1

Changes in the clinical disease activity and MSUS scores over the 6-month abatacept treatment period. The DAS28-CRP (A), SDAI (B), total GS score, and total PD score were significantly improved at 3 and 6 months compared to the baseline. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, Wilcoxon signed ranks test. DAS28-CRP: Disease Activity Score 28-C-reactive protein, GS: grayscale, PD: power Doppler, SDAI: Simple Disease Activity Index.

Figure 2

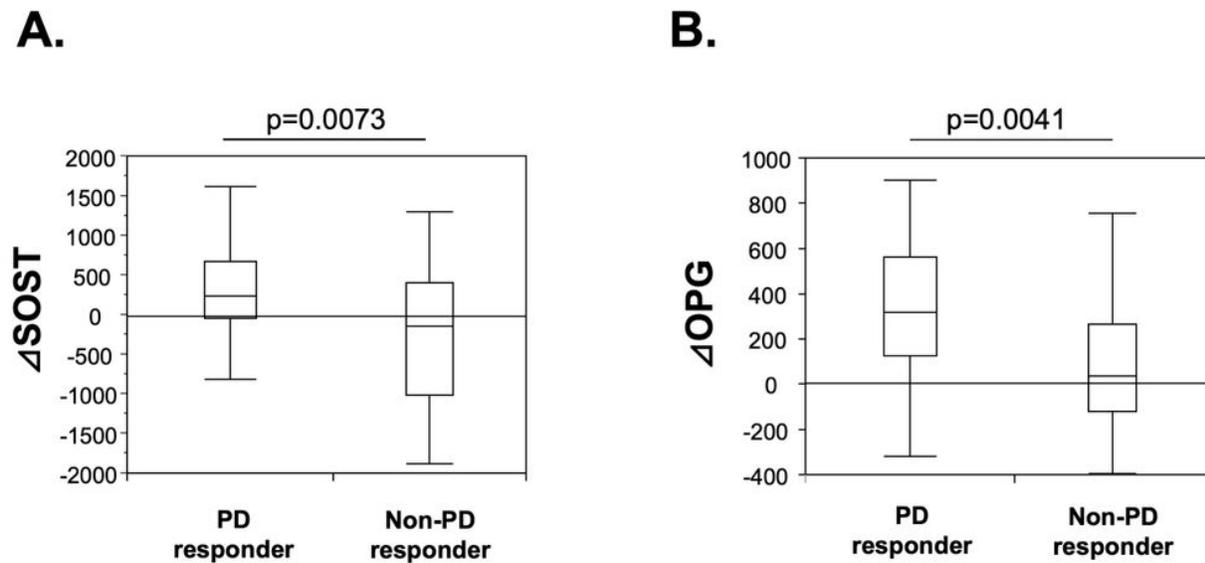


Figure 2

Comparison of Δ SOST and Δ OPG between the PD responders (n=30) and non-PD responders (n=29). Both the Δ SOST and Δ OPG were significantly greater in the PD responders compared to the non-PD responders. Within-group comparisons were made using Mann-Whitney's U-test. Horizontal bar, median; boxes, 25th and 75th percentiles; bars, 5th and 95th percentiles. OPG: osteoprotegerin, SOST: sclerostin.

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