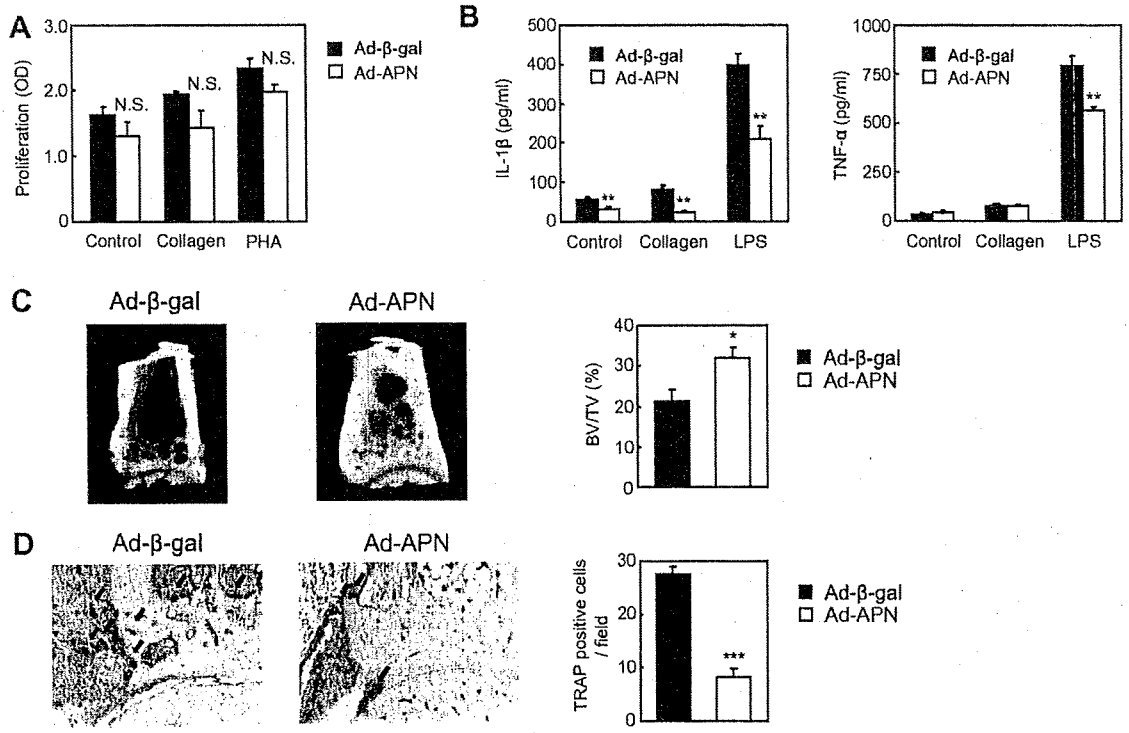

Fig. 4. Effects of Ad-APN on DLN cell proliferation and pro-inflammatory cytokine production from splenocytes, and bone erosion in CIA. All samples were obtained on day 35. (A) Proliferative response of DLN cells obtained from adenovirus-infected CIA mice. Isolated cells from lymph nodes were cultured for 72 h without (control) or with either 50 μg/ml heat-denatured chicken CII or 5 μg/ml phytohemagglutinin (PHA). (B) Production of pro-inflammatory cytokine by splenocytes from adenovirus-infected CIA mice. IL-1β and TNF-α levels were measured in supernatants of splenocytes by specific ELISA. (C) Three-dimensional μCT scan of the distal tibia of adenovirus-infected CIA mice. Trabecular bone volume is expressed as percentage of total tissue volume (BV/TV [%]) ($n = 4$ joints in each group). (D) Reduced number of osteoclasts in Ad-APN-infected CIA mice joints. Sections of ankle joints stained with TRAP (original magnification 100×). The number of TRAP-positive cells was counted in 5 randomly selected fields ($n = 8$ joints in each group). NS = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus Ad-β-gal-infected CIA mice.

APN inhibits osteoclasts differentiation in RAW264 cells [26] and mouse bone marrow macrophages [9]. Collectively, besides anti-inflammatory effects in the joints, Ad-APN might directly inhibits bone erosion of CIA mice by inhibiting osteoclasts differentiation.

The present study demonstrates for the first time that systemic APN delivery provides protection against the development of inflammatory arthritis in a murine model, through several anti-inflammatory mechanisms. The results provide new insights on the role of APN in inflammatory arthritis and new strategies for the treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.11.005.

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ORIGINAL ARTICLE

Serum adiponectin concentrations correlate with severity of rheumatoid arthritis evaluated by extent of joint destruction

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Abstract Adiponectin is a hormone released by adipose tissue with antidiabetic, antiatherogenic, and anti-inflammatory properties. The present observational study focused on the relation between serum adiponectin level and the disease severity of established rheumatoid arthritis (RA). Ninety patients with more than 5-year diagnosis of RA and 42 age- and BMI-matched control were enrolled. The severity of RA was evaluated according to the number of destructed joints of overall 68 joints on plain radiographs (37 patients had mild RA and 53 had severe RA). Serum adiponectin level was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) and mild RA groups (13.9±6.5 μg/ml) (control vs. mild RA group, $P<0.001$; mild RA vs. severe RA group, $P<0.01$). These results suggest that increased number of joint destruction is associated with hyperadiponectinemia in established RA patients.

Keywords Adiponectin · Disease severity · Number of joint destruction · Rheumatoid arthritis

Introduction

Adiponectin is a hormone released by adipose tissue and has various biological properties, such as antidiabetic [1], antiatherogenic [2], and anti-inflammatory effects [3]. Part

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of these effects is mediated by suppressing the production of tumor necrosis factor (TNF)-α and interleukin (IL)-6 by activated macrophage [3]. In addition, it has been reported that adiponectin stimulates the proliferation and differentiation of human osteoblasts [4] and suppresses the differentiation of osteoclasts [5], suggesting that adiponectin may play a role in rheumatoid arthritis (RA).

A recent clinical study showed that serum adiponectin concentrations are higher in RA patients than in healthy control [6, 7]. In addition, adiponectin induces the production of pro-inflammatory IL-6 from RA synovial fibroblasts in vitro [8], suggesting that adiponectin is a potent driving force of arthritis. On the other hand, another report demonstrated that adiponectin concentrations correlated negatively with the number of leukocytes in the synovial fluid of RA patients [7], indicating that adiponectin is a counterpart of the local inflammatory process. Thus, the role of adiponectin in RA is controversial. In a step to define the role of adiponectin in RA, the present study was designed to investigate the correlation between serum adiponectin level and RA disease severity.

Materials and methods

Patients

We have previously reported that serum adiponectin level is significantly higher in females than in males and negatively correlates with body mass index (BMI) [9]. In addition, previous reports have demonstrated that most of the progression of joint damage in RA occurs during the first years of the disease and decreases thereafter [10, 11]. Therefore, to investigate the correlation between serum adiponectin level and disease severity and joint destruction in established RA, 90 female patients with more than 5-year history of RA were enrolled in this study. RA was diagnosed based on the 1987 revised American College of Rheumatology (ACR) criteria [12]. The first assessment was carried out from September to November, 2005, and 18 patients were enrolled in the second assessment from March to April, 2008, about 2.5 years after the first assessment. Sixty-five patients (72.2%) were treated with oral prednisolone and 48 patients (53.3%) with methotrexate. All patients were followed-up at Osaka University Hospital.

For non-RA controls, 42 age- and BMI-matched women who underwent health examination at the institutions that participated in the Japanese Visceral Fat Syndrome (J-VFS) Study Committee of the Ministry of Health and Welfare of Japan and subjects who visited Osaka University Hospital for health check were enrolled in the present study [13]. Patients treated with antihypertensive, antidiabetic, or antihyperlipidemic regimen or patients who met the definition of each disease indicated in the relevant guidelines were defined as having hypertension, diabetes, and hyperlipidemia, respectively. Patients treated with drugs influencing serum adiponectin levels, such as anti-TNF-α [14, 15], insulin [16], thiazolidinediones [17], telmisartan [18], glimepiride [19], and all other biologics were excluded in this study. The study was approved by the Ethical Committee of Osaka University School of Medicine and written informed consent was obtained from each patient.

Assessment of disease severity and disease activity

The severity of RA was evaluated by the number of joints with erosions among 68 joints of whole body using plain radiographs, as described previously [20]. Joint erosion was defined as changes equal to or more severe than stage II according to the criteria of Steinbocker et al. [21]. Patients were classified according to disease severity as described previously [22]. Briefly, the least erosive subset (LES) group exhibited erosions in less than 20 joints and erosive articular changes limited to the small peripheral joints of hands or feet. The more erosive subset (MES) group had erosions in more than 21 joints and erosive articular changes in large axial joints. The most erosive subset with mutilating disease (MUD) group, that had erosions in more than 46 joints, and almost all joints were extensively damaged in the early period of RA. In this study, we categorized LES patients as the "mild RA group" (n=37), and MES/MUD patients as the "severe RA group" (n=53). Disease activity score including a 28 joint count/CRP (DAS28-CRP) was evaluated as described previously [23].

Measurement of serum adiponectin concentrations

Total serum adiponectin level (including all isoforms) was measured with an enzyme-linked immunosorbent assay (ELISA) kit (Otsuka Pharmaceutical, Tokyo, Japan), as reported previously [13].

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences in variables between the mild and severe RA groups were assessed by the Mann–Whitney U test and the chi-square test. Changes in serum adiponectin levels between the first and second assessment was examined by the Wilcoxon's signed rank test. The influence of serum adiponectin level on other variables was investigated by calculating Spearman's correlation coefficients. The correlation between BMI and disease severity was investigated by logistic regression analysis. Conditional multivariate logistic regression models were constructed and odds ratios (ORs) and 95% confidence intervals (95% CI) were cal-

culated to investigate the association of serum adiponectin level on disease severity, with adjustment for BMI. To investigate the cutoff value for serum adiponectin, a value yielding 80% correspondence to the severity of RA was estimated by a logistic regression model and statistical significance was estimated by Fisher's exact test. Probability values of less than 0.05 were considered statistically significant. All statistical analyses were carried out with SAS software version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Clinical and biochemical characteristics of the study subjects

There were no significant differences between mild and severe RA groups in age (60.8±11.0 vs. 61.7±11.7 years), disease duration (15.5±6.9 vs. 17.3±6.8 years), body mass index (22.1±3.4 vs. 20.8±3.0 kg/m^2), and prevalence of hypertension (18.8% vs. 28.3%), diabetes (16.2 vs. 18.9%), and hyperlipidemia (21.6 vs. 24.5%). The age and BMI of subjects of the control group were 61.0±11.4 years and 21.9±3.2 kg/m^2, respectively. The prevalence of each

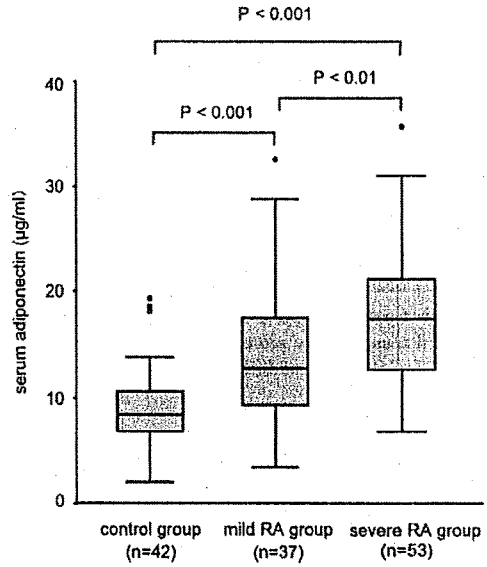

Fig. 1 Box-and-whisker plots of serum adiponectin levels in the control group, mild RA group, and severe RA group evaluated by the number of joint destruction in 68 joints on plain radiograph. The mean serum level of adiponectin was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) or mild RA group (13.9±6.5 μg/ml) (control vs. mild RA group: $P<0.001$, mild RA vs. severe RA group: $P<0.01$, control vs. severe RA group: $P<0.001$)

Table 1 Baseline demographic, laboratory, and clinical characteristics of the two RA groups

	mild RA group (n=37)	severe RA group (n=53)	P[a] value
Age, years	60.8±11.0	61.7±11.7	NS
Duration of disease, years	15.5±6.9	17.3±6.8	NS
Body mass index, kg/m^2	22.1±3.4	20.8±3.0	NS
CRP, mg/l	0.9±1.3	1.9±2.0	0.003
MMP-3, ng/ml	146.5±150.9	222.8±177.6	0.047
IL-6, ρg/ml	14.5±36.0	18.1±26.2	NS
RF titer, IU/ml	158.1±417.0	235.7±366.9	NS
RF positivity, % patients	77.1%	82.7%	NS[b]
BAP, U/l	24.0±13.5	23.9±9.5	NS
iOC, ng/ml	6.9±3.5	6.7±7.3	NS
1CTP, ng/ml	4.9±1.9	6.5±3.1	0.010
uDPD, nmol/mmol creatinine	6.6±2.2	7.9±3.2	NS
DAS28-CRP	2.2±1.0	3.1±1.5	0.001
Prednisolone dosage, mg/day	2.2±2.5	4.3±3.6	0.001
Methotrexate dosage, mg/week	4.3±3.2	4.4±3.6	NS
Adiponectin, μg/ml	13.9±6.5	17.7±6.7	0.008

Data are mean ± SD

RA rheumatoid arthritis, *NS* not significant, *CRP* C-reactive protein, *MMP-3* matrix metalloproteinase-3, *IL-6* interleukin-6, *RF* rheumatoid factor, *BAP* bone-specific alkaline phosphatase, *iOC* intact osteocalcin, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *uDPD* urinary deoxypyridinoline, *DAS28-CRP* disease activity score including a 28-joint count/CRP

[a] Except where otherwise indicated, determined by Mann–Whitney U test

[b] Except where otherwise indicated, determined by chi-square test

Table 2 Spearman's correlation analysis of the relation between serum adiponectin and other variables in all RA patients

Variable	r value	P value
Age, years	0.046	NS
Duration of disease, years	0.068	NS
Body mass index, kg/m^2	−0.269	0.011
CRP, mg/liter	0.078	NS
MMP-3, ng/ml	0.098	NS
IL-6, ρg/ml	0.120	NS
RF titer, IU/ml	−0.033	NS
BAP, U/l	−0.193	NS
iOC, ng/ml	−0.075	NS
1CTP, ng/ml	0.033	NS
uDPD, nmol/mmol creatinine	−0.002	NS
DAS28-CRP	0.096	NS
Prednisolone dosage, mg/day	0.040	NS

r value Spearman's rank correlation coefficient, *NS* not significant, *CRP* C-reactive protein, *MMP-3* matrix metalloproteinase-3, *IL-6* interleukin-6, *RF* rheumatoid factor, *BAP* bone-specific alkaline phosphatase, *iOC* intact osteocalcin, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *uDPD* urinary deoxypyridinoline, *DAS28-CRP* disease activity score including a 28-joint count/CRP

Table 3 Results of Spearman's rank correlation analysis of the relation between adiponectin and other variables with a significant difference between the severe and mild RA groups

	CRP	MMP-3	1CTP	DAS28-CRP	Prednisolone	Adiponectin
CRP		0.574***	0.486***	0.717***	0.335**	0.078
MMP-3			0.331**	0.532***	0.510***	0.098
1CTP				0.389***	0.220*	0.033
DAS28-CRP					0.372**	0.096
Prednisolone						0.040

CRP C-reactive protein, *MMP-3* matrix metalloproteinase-3, *1CTP* pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen, *DAS28-CRP* disease activity score including a 28-joint count/CRP
*$P<0.05$; **$P<0.01$; ***$P<0.001$

disease in the control group was 0% for hypertension, 2.3% for diabetes, and 9.5% for hyperlipidemia. Patients of the severe RA group had a significantly higher serum C-reactive protein (CRP) ($P=0.003$), matrix metalloproteinase (MMP)-3 ($P=0.047$), pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP) ($P=0.010$), disease activity score including a 28-joint count/CRP (DAS28-CRP) ($P=0.001$), and prednisolone dose ($P=0.001$) than the mild RA group (Table 1), reflecting high inflammatory state and bone resorption level in this group as described previously [24]. The mean serum level of adiponectin was significantly higher in the total RA group (16.1±6.8 μg/ml) than in the control group (9.1±3.8 μg/ml) ($P<0.001$). Moreover, the mean serum level of adiponectin was significantly higher in the severe RA group (17.7±6.7 μg/ml) than in the control (9.1±3.8 μg/ml) or mild RA group (13.9±6.5 μg/ml) (control vs. mild RA group, $P<0.001$; mild RA vs. severe RA group, $P<0.01$, control vs. severe RA group, $P<0.001$) (Fig. 1). Univariate analysis of the relationship between serum adiponectin level and other variables showed that adiponectin correlated negatively with BMI ($r=-0.269$, $P=0.011$), but did not correlate with other variables such as inflammatory markers, bone metabolism markers, DAS28-CRP, or the dose of prednisolone (Table 2). Calculation of Spearman's rank correlation coefficients for the variables with a significant difference between the mild and severe RA groups showed that CRP correlated with MMP-3 ($r=0.574$, $P<0.001$), 1CTP ($r=0.486$, $P<0.001$), DAS28-CRP($r=0.717$, $P<0.001$), and dose of prednisolone ($r=0.335$, $P<0.01$), while there was no significant correlation with adiponectin ($r=0.078$, $P>0.05$) (Table 3). In addition, the dose of prednisolone correlated with CRP, MMP-3, 1CTP, and DAS28-CRP, but not with adiponectin ($r=0.040$, $P>0.05$) (Table 3). Multivariate logistic regression analyses revealed that even when the odds ratios were adjusted for BMI, serum adiponectin level significantly correlates with disease severity of RA ($P=0.031$) (Table 4).

Cutoff point of serum adiponectin for severe RA

Figure 2 shows the histogram of serum adiponectin levels of patients of the mild and severe RA groups. For clinical translation, the cutoff levels were selected. The cutoff value for serum adiponectin level was estimated at 18 μg/ml, yielding 80% correspondence with the severity of RA. Among the patients with serum adiponectin level of ≥18 μg/ml, 81.3% (26/32) belonged to the severe RA group and 18.8% (6/32) belonged to the mild RA group. This cutoff line showed significant correlation with disease severity ($P<0.01$). The specificity of this cutoff value was 53.4% (31/58) (Table 5).

Changes in serum adiponectin levels and severity of RA during follow-up

To further investigate the time-course changes of serum adiponectin levels and severity of RA, 18 patients underwent a second assessment 2.5 years later (Fig. 3). The mean serum adiponectin level of all patients did not change significantly, although it showed tendency to increase (14.0±5.5 to 15.2±5.2 μg/ml; $P=0.07$). Furthermore, the mean serum adiponectin level did not change significantly within the RA group (10.8±5.8 to 12.4±5.8 μg/ml in the mild RA group, $P=0.122$ and 17.2±2.7 to 18.0±2.4 μg/ml in the severe RA group, $P=0.372$). Assessment of RA severity revealed that none of the mild RA patients progressed to severe RA (data not shown).

Table 4 Adjusted ORs of serum adiponectin level and BMI for disease severity of RA

	Adjusted OR	95% CI	*P* value
Adiponectin, μg/ml	1.085	1.007–1.168	0.031
BMI, kg/m^2	0.907	0.785–1.048	NS

ORs odds ratios, *95% CI* 95% confidence interval, *NS* not significant

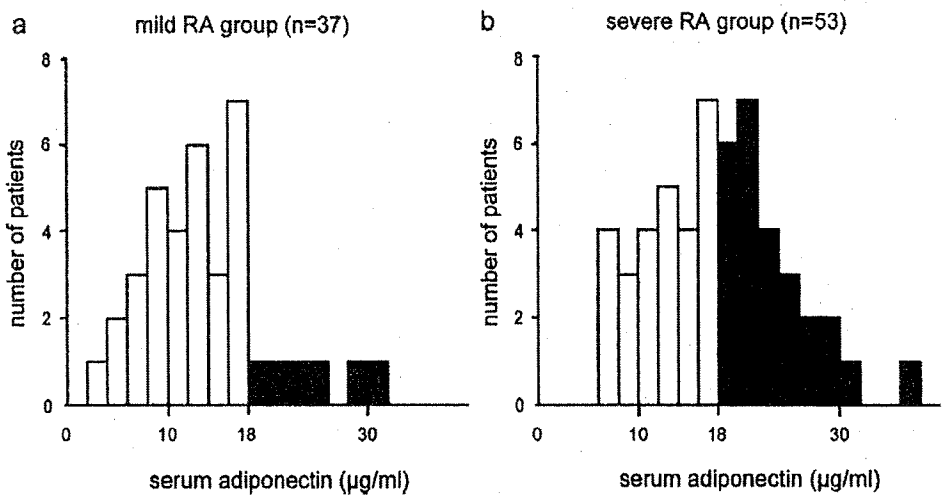

Fig. 2 Histograms showing the distribution of serum adiponectin levels in mild RA group (a) and severe RA group (b). Each column covers a serum adiponectin range of 2 μg/ml. When the cutoff value for adiponectin was set at 18 μg/ml, there was 80% correspondence with the severity of RA. Among patients with serum adiponectin levels ≥18 μg/ml, 81.3% (26/32) belonged to the severe RA group and 18.8% (6/32) belonged to the mild RA group

Discussion

The long-term functional prognosis of RA patients in daily life is mainly determined by the extent of damage in large joints such as the hip, knee, ankle, subtalar, shoulder, and elbow joints, rather than in small joints of the hands or feet. A previous report using Ochi's method demonstrated that MES and MUD groups underwent higher frequency of total knee or hip replacement than LES group (54.7% vs. 0.5%) [25], suggesting that Ochi's method offers some advantages for assessing large joint destruction [20, 25–27]. Therefore, we used Ochi's method to evaluate the severity of RA, to investigate the factors associated with the extent of overall joint destruction, especially in large joints [20]. Evaluation using this method revealed that markers associated with RA activity, such as CRP, MMP-3, 1CTP, and DAS28-CRP were all significantly higher in the severe RA group than in the mild RA group. These results were in agreement with previously published reports evaluated by the modified Sharp/van der Heijde method and Larsen's method (CRP [28], MMP-3 [29], 1CTP [30], and DAS [31]).

We showed for the first time that serum adiponectin levels were higher in the severe RA group than in control and mild RA groups. Interestingly, while other disease severity-related variables, such as MMP-3, 1CTP, DAS28-CRP, and dose of prednisolone correlated with CRP, serum adiponectin levels did not, in both the mild and severe RA groups (Table 3). It has been reported that serum TNF-α and CRP levels are elevated in RA patients [32, 33], and TNF-α, CRP, and corticosteroid markedly inhibit adiponectin gene expression in cultured adipocytes [16, 34]. Furthermore, anti-TNF-α therapy restored serum adiponectin level in RA patients [14, 15, 35]. On the other hand, despite elevated CRP levels and higher dose of treated oral prednisolone (corticosteroid), serum adiponectin levels were elevated in the severe RA group than in the mild

Table 5 Separation of mild and severe RA using a cutoff value for serum adiponectin of 18 μg/ml

	Serum adiponectin level	
	<18 μg/ml	≥18 μg/ml
Mild RA group (n)	31	6
Severe RA group (n)	27	26
% with severe RA	46.6%	81.3%

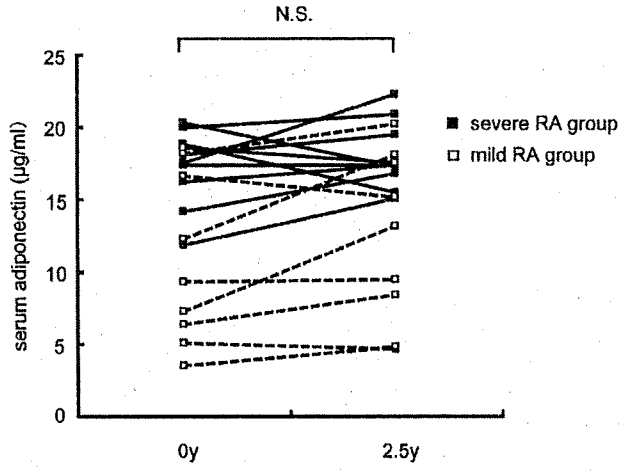

Fig. 3 Changes in serum adiponectin levels of 18 RA subjects in 2.5-year interval. The mean serum adiponectin level of the whole group was 14.0±5.5 μg/ml at baseline (0 years) and 15.2±5.2 μg/ml at follow-up (2.5 years, P=0.07); 10.8±5.8 vs. 12.4±5.8 μg/ml in mild RA group (P=0.122) and 17.2±2.7 vs. 18.0±2.4 μg/ml in severe RA group (P=0.372) and the change was not significant in either groups. None of the mild RA patients showed worsening to severe RA category during this period

RA group in the present study (Table 3). Considered together, serum adiponectin should be induced by unknown factors associated with the number of destructed joints, especially in large joints, and their effects should exceed the inhibitory effects of TNF-α, CRP, and prednisolone in RA patients. Recently, Fantuzzi [36] suggested that adiponectin promotes survival during periods of catabolism secondary to malnutrition and that hyperadiponectinemia may be the result of response to catabolic state in RA. Consequently, a catabolic state accompanied by joint destruction, especially in large joints, may be one of the strong inducer of serum adiponectin level.

It has been reported that a low BMI is a sensitive and independent predictor of radiographic progression of joint damage assessed by Larsen's method in RA [37, 38]. In this study, there was no significant difference in BMI between control, mild RA, and severe RA group (21.9±3.2 vs. 22.1± 3.4 vs. 20.8±3.0 kg/m^2). In addition, in total RA group, BMI showed only tendency of positive correlation with disease severity (P=0.059). However, under such condition, serum adiponectin levels were significantly higher in severe RA group than in the control and mild RA groups (Table 1, Fig. 1). Furthermore, multivariate logistic regression analyses revealed that even when the odds ratios were adjusted for BMI, serum adiponectin level significantly correlates with disease severity of RA (Table 4). Therefore, we speculate that serum adiponectin levels can be a better sensitive indicator of the expansion of joint destruction than BMI in RA patients. To further investigate the time course changes in serum adiponectin levels and severity of RA, 18 patients were assessed 2.5 years later. The results showed no significant change in serum adiponectin levels and none of the mild RA patients progressed to severe RA. The lack of change in the severity category during this period is compatible with previous reports indicating that most of the progression of joint damage in RA occurs during the first years of the disease and decrease thereafter [10, 11, 20]; and under such conditions, serum adiponectin level is relatively stable in established RA (disease duration ≥5 years).

For clinical translation of these findings, we determined the cutoff level of serum adiponectin level. The estimated cutoff levels estimated by the histogram of serum adiponectin level showed relatively high sensitivity (81.3%) but low specificity (53.4%) in this study (Fig. 2, Table 5), indicating that increased number of destructed joints may be one of the additive, but not a specific factor of high serum adiponectin level in RA. Consequently, prospective studies in early stage of RA (disease duration <5 years) and in large number of RA patients are needed to determine the cutoff level of adiponectin to be used as an indicator or predictor of destructed joints. In addition, to elucidate the effect of hyperadiponectinemia on the severity of RA, further animal experiments are needed.

Despite the limitation of observational study, we demonstrated that the severity of RA, evaluated by the number of destructed joints on plain radiographs detected in the whole skeleton, correlated with serum adiponectin concentrations. This finding should encourage further research to investigate the role of adiponectin in RA and design new adiponectin-based treatment strategies for RA.

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Disclosures None.

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Morphologic analysis of the medullary canal in rheumatoid elbows

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Summary Total elbow arthroplasty is a standard approach for patients with arthritic elbows. To design appropriate stems for elbow prostheses, it is important to understand the shape of the medullary canals. The purpose of this study was to evaluate the shape and size of the medullary canals from normal cadavers and rheumatoid arthritis patients. These canals were measured based on geometric constructions of the 3-dimensional bone models generated from computed tomography images. The cross-sectional area of the medullary canals in rheumatoid arthritis patients decreased near the elbow joint as a result of morphologic changes after a long-standing inflammatory reaction. When designing the press-fit component of the humerus, an increase in the width of the transverse diameter of the intramedullary stem could increase stability in the canal. In contrast, for the ulnar component, such morphologic changes would impose difficulty in placing the press-fit model despite an anatomically designed stem. Therefore, a cement technique would be required for improved stabilization of the ulnar component.

Rheumatoid arthritis (RA) is an inflammatory condition, typified by synovial proliferation, that can affect multiple joints, with elbow involvement being the most common in nearly half of RA cases.[12] Total elbow arthroplasty (TEA) in rheumatoid patients is now a widely accepted therapeutic alternative for advanced elbow destruction. The advantage of TEA over simple synovectomy is that it can reduce pain, improve the range of joint motion, and provide long-term pain relief. However, loosening of the implants is one of the major complications and a primary concern. Although the improvement in surgical technique and prosthetic design has led to increased success with TEA, high rates of humeral component loosening have been reported previously.[5,8,18,23,26] Stem design of the prosthesis is considered an important factor that influences loosening of the implant. Data on the geometry of the medullary canals of the distal humerus and proximal ulna should help to design stems for elbow prostheses, which could occupy the medullary cavity, increase its stability with regard to the bone, and decrease the rate of loosening. However, in these patients, it is difficult to evaluate the morphologic analysis appropriately, based only on plain radiographs. In addition, RA patients have shown a restricted range of motion of the elbow with occasional valgus deformity because of erosive changes. Therefore, with only an axial computed tomography (CT) scan, it is difficult to evaluate the morphology accurately. Recent advancements in computer technology have enabled us to determine the precise geometry of the medullary canals generated from the CT data. We have

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constructed 3-dimensional (3D) bone models and resliced the medullary canal vertically along its longitudinal axis to obtain accurate measurements. The purpose of this study was to evaluate the geometry of the medullary canals of the distal humerus and proximal ulna using 3D bone models constructed from the CT data and to compare them between normal cadavers and RA patients.

Materials and methods

We studied the elbow joints of 16 seropositive rheumatoid patients, who were scheduled for TEA (15 women and 1 man; age range, 48-81 years; mean age, 60.6 years) and 20 cadaveric dry bones (10 humeri and 10 ulnae) (Natural Bone; Sawbones [A Division of Pacific Research Laboratories], Vashon, WA). The inclusion criteria for RA patients included RA of grade III to V based on the radiographic criteria of Larsen et al,[10] severe disabling pain, limitation of elbow function, and no previous surgical treatment. Of the patients, 9 (56.2%) had Larsen grade III RA, 4 (25%) had Larsen grade IV RA, and 3 (18.8%) had Larsen grade V RA. The right elbow was affected in 10 patients and the left elbow in 6.

Image acquisition

Morphologic analysis of the medullary canal was performed by use of CT scans. Image data of the elbows from RA patients and cadavers were obtained with a helical CT system (Light Speed Ultra16; GE Medical Systems, Waukesha, WI). We used a sequence with 120 kV, 100 mA, a 300-mm field of view, and a thickness of 0.625 mm on a contiguous slice with a pixel size of 0.39 × 0.39 mm. During CT scanning, the elbow joint was maximally extended with the forearm in a neutral position. Scan data were saved in DICOM (Digital Imaging and Communications in Medicine) format.

Three-dimensional bone models and measurement

Contours and medullary structures of each bone were semiautomatically segmented from CT images by use of the Virtual Place-M software program (Medical Imaging Laboratory, Tokyo, Japan). This software generated 3D surface bone models via the marching cubes technique,[13] based on which we constructed bone models of each humerus and ulna. A threshold for constructing these models is an important parameter for determining their accuracy. In this study, we used 150 Hounsfield units as an optimal threshold value.[21] The contours of the medullary canal were extracted semiautomatically, and the visualization and measurement of the geometric shape and size of each bone were obtained by use of a visualization software program developed in our laboratory. The accuracy of this computer-based measurement was reported to be 0.4 mm.[17]

We resliced the 3D surface bone models of the medullary canal vertically along the longitudinal axis of each canal and evaluated the change in medullary shape of the reconstructed cross section of the humeral medullary canal, from the proximal margin of the olecranon fossa, and for the ulna, from the tip of the coronoid process, at intervals of 1 cm. Then, we calculated the area, as well as the anteroposterior and transverse diameters, of each cross-sectional slice of the medulla of the humerus and the ulna.

Statistical analysis

Mann-Whitney *U* tests were performed for statistical differences between normal controls and RA patients. A difference with $P < .05$ was considered significant. This statistical analysis was conducted with the Statcel2 statistical analysis software package for personal computers (OMS Publishing, Saitama, Japan).

Results

Morphology of humerus

The cross-sectional shape of the humerus changed from a V shape to an equilateral triangle and then to ovoid in both RA patients and cadavers (Figure 1, *A*). The cross-sectional area particularly decreased at 0 cm above the olecranon fossa compared with the cadavers ($P < .05$). It also tended to decrease at a distance of 1 cm (Figure 2, *A*). Whereas the mean anteroposterior diameter was not significantly different between RA patients and controls, the mean transverse diameter was significantly decreased at 0 and 1 cm in the RA patients as compared with the controls ($P < .05$), as shown in Figures 2, *B*, and 2, *C*. In RA patients and cadavers, the cross-sectional areas were approximately 100 mm^2 at 2 cm and 80 mm^2 at 3 to 10 cm and then they increased gradually at 11 cm. The mean anteroposterior diameters of the humerus tended to increase gradually toward the diaphysis, whereas the transverse diameters gradually decreased.

Morphology of ulna

For the ulna, the shape changed from ovoid to triangular (Figure 1, *B*), and the cross-sectional area decreased at 0 and 1 cm distal to the coronoid process in the RA patients ($P < .05$) (Figure 3, *A*). In the ulna, both the mean anteroposterior and transverse diameters were significantly decreased at 0 and 1 cm, respectively, in the RA patients compared with the controls ($P < .05$) (Figures 3, *B*, and 3, *C*). This discrepancy between RA patients and cadavers was produced by the morphologic changes observed around the trochlear notch of the ulna. In both RA patients and cadavers, the cross-sectional areas were approximately 50 mm^2 at 3 cm and 20 mm^2 at 7 cm to 14 cm. Both the mean anteroposterior and transverse diameters of the ulna tended to decrease gradually toward the diaphysis.

Discussion

TEA can preserve, or even improve, the range of motion of the joints, eliminate pain, and provide adequate stability to the arthritic joint. One major concern regarding TEA is loosening of the implants. Although improvement of surgical technique and prosthetic design has led to

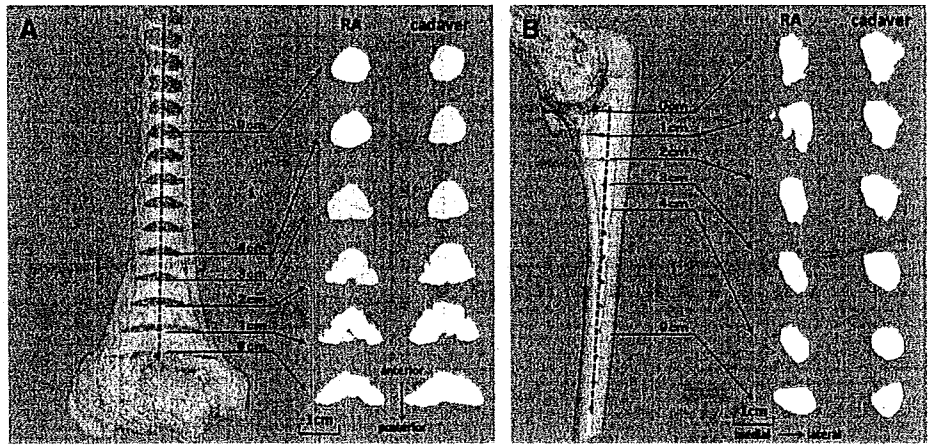

Figure 1 (A) Cross-sectional area of medullary canal of humerus. We evaluated the cross-sectional area from the proximal margin of the olecranon fossa at 1-cm intervals. The cross-sectional shape of the humerus changed from a V shape to an equilateral triangle and then to ovoid in both RA patients and cadavers. The cross-sectional area of RA has especially decreased at 0 cm above the olecranon fossa because of the morphologic change around the coronoid fossa, as compared with the cadavers ($P < .05$). (B) Cross-sectional area of medullary canal of ulna. The geometric shape of the cross-sectional area changed from ovoid to triangular. In the RA patients, the cross-sectional area decreased at 0 and 1 cm distal to the tip of the coronoid process because of the morphologic change around the trochlear notch of the ulna.

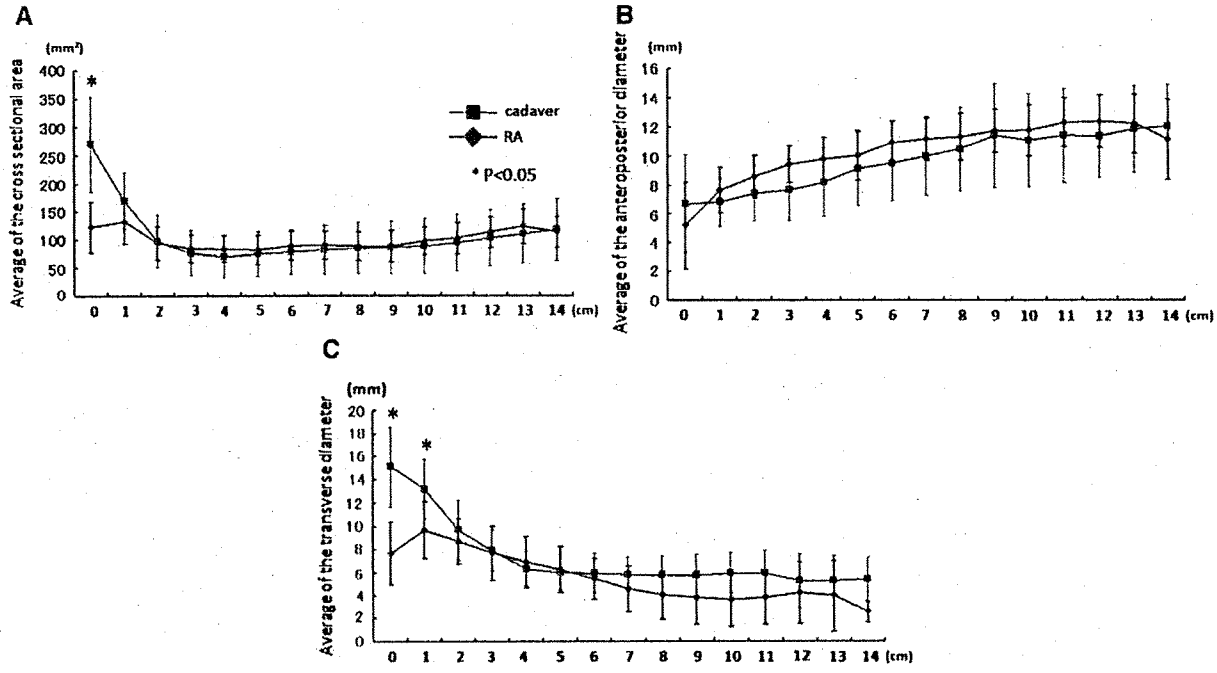

Figure 2 Measurement of medullary canal of humerus. (A) The mean cross-sectional area of the humerus is shown with SD error bars. *Asterisk*, A significant difference was found between the RA patients and cadavers ($P < .05$). (B) Mean anteroposterior diameters of cross section. (C) Mean transverse diameters of cross section.

increased longevity of TEA, the loosening rate is still higher when compared with total hip and knee arthroplasties.[5,6,8,18,19,23,26] In RA patients, van der Lugt et al[26,27] reported that the survival rate of the Souter-Strathclyde prosthesis, according to Kaplan-Meier analysis, was 77.4% after 10 years and 65.2% after 18 years. A high rate of aseptic loosening of the humeral component has been frequently described, whereas the success rate was found to be different among the types of TEA.[5,8,18,23,26] Although several authors postulate that the loosening was because of the micromovement, the reason for this phenomenon remains unclear.[2,24] Ikavalko et al[3,4] reported that the standard stem humeral component has better survivorship compared with the long-stemmed component.

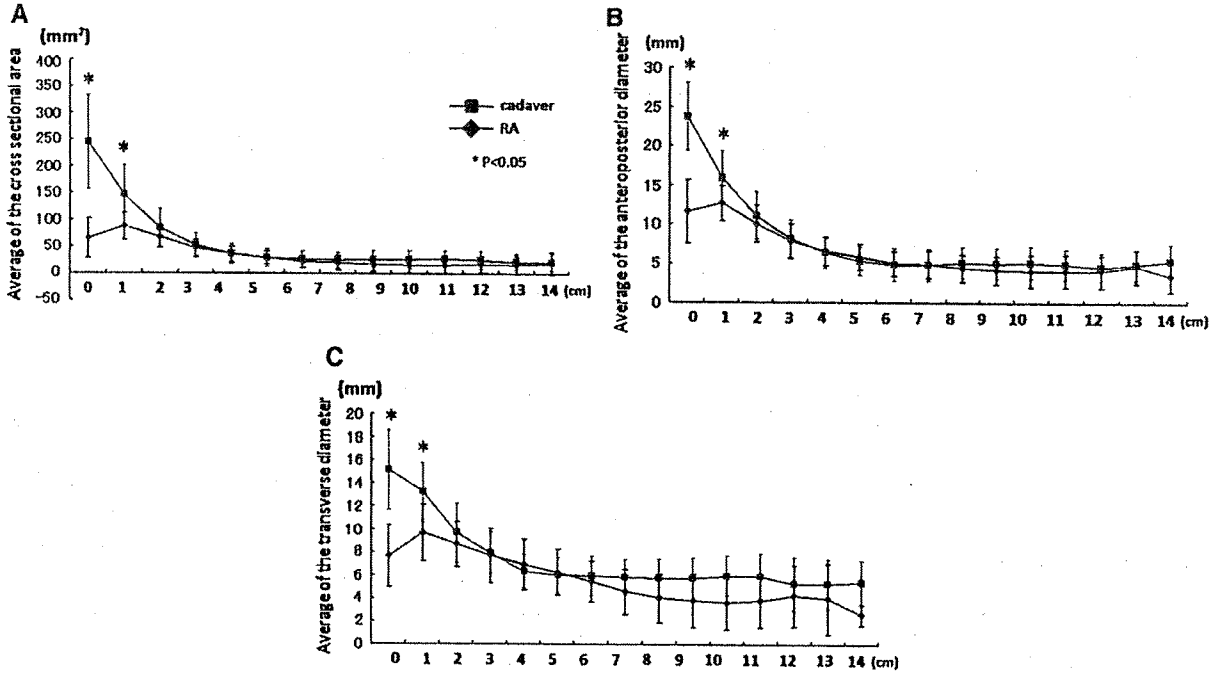

Figure 3 Measurement of medullary canal of ulna. (A) The mean cross-sectional area of the ulna is shown with SD error bars. *Asterisk*, A significant difference was found between the RA patients and cadavers ($P < .05$). (B) Mean anteroposterior diameters of cross section. (C) Mean transverse diameters of cross section.

We assumed that the morphologic change of periarticular inflammatory reaction from arthritis could possibly be one of the reasons for early aseptic loosening after TEA, as it can decrease the stability of the implant in regard to the bone. Therefore, it would be important to evaluate the morphologic change around the elbow joint in RA patients accurately. There have been several detailed studies of the geometry of the medullary canals based on radiographs.[7,11,20] However, it is quite difficult to quantify the extent of the bone loss correctly, based only on plane radiographs.

On the other hand, several authors believe that an anatomic, custom-designed femoral component of total hip arthroplasty is more likely to be effective than off-the-shelf components to achieve the optimal fit and fill the medullary canal.[14,15,28,29] The geometry of the medullary canals of the distal humerus and proximal ulna is considered important for the appropriate design of stems for the TEA prosthesis to increase its stability in the bone and decrease the rate of loosening. Our measurements were derived from 3D constructions of the medullary canal, based on the CT data obtained with a high-speed helical scanner. This allowed us to compare the morphology of the normal controls with the RA group accurately.

High loosening rates of the humeral component have been reported previously.[5,8,18,23,26] On the basis of the data obtained from this study, the anteroposterior diameters of the humerus have tended to increase toward the diaphysis whereas the transverse diameters have decreased (Figure 2, *B*). When considering the initial fixation of the press-fit uncemented stem of the humeral component in future prosthetic design, the width of the transverse diameter is considered more important than the anteroposterior diameter. Increasing the width of the transverse diameter of the intramedullary stem could increase the stability of the humeral component in the canal.

Several authors have noted that aseptic loosening and failure, because of loosening, occur more often with the ulnar component than with the humeral component and that the ulnar component is at high risk of loosening.[1,9,16,22,25] Furthermore, in RA patients, aseptic loosening of the ulnar component was found more often in uncemented ulnar components than in cemented ones. Our results showed that there is a significant morphologic change in the proximal ulnar medullary canal in the area from the coronoid process to 3 cm distally, where the stem of the prosthesis is expected to fit. This change in the morphology would make press fitting of the ulnar component into the canal quite difficult, even with an anatomically designed stem. We believe that the ulnar component would be better stabilized by a cement technique, especially in cases of severe morphologic change.

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ORIGINAL ARTICLE

A combination of biochemical markers of cartilage and bone turnover, radiographic damage and body mass index to predict the progression of joint destruction in patients with rheumatoid arthritis treated with disease-modifying anti-rheumatic drugs

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Abstract The aim of this study was to evaluate the predictive value of biological, radiological and clinical parameters for the progression of radiographic joint damage in rheumatoid arthritis (RA) patients treated with conventional disease-modifying anti-rheumatic drugs (DMARDs). We analyzed the 145 patients with active RA for less than 5 years who were participating in the prospective 1-year randomized controlled trial of tocilizumab (SAMURAI trial) as a control arm treated with conventional DMARDs. Progression of joint damage was assessed by sequential radiographs read by two independent blinded X-ray readers and scored for bone erosion and joint space narrowing (JSN) using the van der Heijde-modified Sharp method. Multivariate analysis revealed that increased urinary levels of C-terminal crosslinked telopeptide of type II collagen (U-CTX-II), an increased urinary total pyridinoline/total deoxypyridinoline (U-PYD/DPD) ratio and low body mass index (BMI) at baseline were independently associated with a higher risk for progression of bone erosion. In addition to these three variables, the JSN score at baseline was also significantly associated with an increased risk of progression of the JSN score and total Sharp score. High baseline U-CTX-II levels, U-PYD/DPD ratio and JSN score and a low BMI are independent predictive markers for the radiographically evident joint damage in patients with RA treated with conventional DMARDs.

Keywords BMI · CTX-II · Joint destruction · PYD/DPD ratio · Rheumatoid arthritis

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Introduction

Although rheumatoid arthritis (RA) has features of a systemic disease and capable of exhibiting a variety of extra-articular manifestations, it is predominantly characterized by structural destruction of the joints, leading to functional disability [1–4]. Joint destruction often progresses early in the disease process [5–8], but the process is highly variable from patient to patient [9–12]. The identification of patients with rapid joint destruction very early in the disease process is of critical importance to clinicians wanting to optimize treatment strategies. Indeed, although new biological therapies are highly effective in preserving joint structure, they are expensive and may have side effects.

Thus, targeting these treatments to RA patients manifesting rapid progression of the disease may be beneficial.

Several prospective studies have been performed to identify predictive factors indicative of a worse radiological progression of RA [13–31]. The earlier investigations revealed the importance of the rheumatoid factor (RF), inflammation markers or radiographic damage at baseline [13, 14, 16–18, 20, 21], while more recent ones have identified biochemical markers of bone, cartilage and synovial tissue metabolism and catabolic enzymes as being associated with progression in RA [15, 19, 22, 24, 27–29]. Alternatively, RA is also associated with accelerated atherosclerosis and increased cardiovascular mortality and, recently, it has been shown that macrophage inhibitory cytokine 1 (MIC-1), which is linked to clinical events in atherosclerosis, may be involved in the pathological process of erosive joint destruction [32]. The body mass index (BMI) has also been reported to be associated with the radiographic progression of RA, independent of inflammation markers [23, 30, 31], and recent new information suggests the potential involvement of adipokines as regulators of inflammation in RA [33]. These new findings have lead to the recognition of RA as a disease involving a variety of pathological conditions related with joint destruction and made clinicians aware of the fact that RA is a systemic disease in terms of the pathology of the bone and destruction of cartilage. However, to date, there has been no study that has analyzed concomitantly in the same population the independent contribution of these various anthropometric, clinical, laboratory and radiological features to the prediction of disease progression in RA.

The aims of the study reported here were to determine which combination of a few risk factors identified among a panel of clinical, biological and radiological parameters would be powerful in predicting the radiological progression of bone erosion and joint space narrowing (JSN) in RA patients treated with conventional disease-modifying antirheumatic drugs (DMARDs).

Methods

Patients and protocol

The patient cohort consists of 148 patients with RA receiving conventional DMARDs who participated in the control arm of the SAMURAI trial described in a recent publication [34]. The aim of the SAMURAI, which was a 52-week-long multi-center clinical trial, was to evaluate the effect of tocilizumab on radiological joint damage. Three hundred and six patients with RA diagnosed according to the American College of Rheumatology criteria [35] were randomly assigned to tocilizumab monotherapy (8 mg/kg intravenously every 4 weeks) or conventional DMARDs. For the DMARDs group, the dose, type and combination of DMARDs and/or immunosuppressants could vary according to disease activity at the discretion of the treating physician. The study protocol was approved by the Ministry of Health, Labor and Welfare of Japan, and by the ethical committee at each participating site, and patients gave their written informed consent.

Radiographic assessment

Posteroanterior radiographs of hands and anteroposterior radiographs of feet were performed at baseline and at weeks 28 and 52 or at the last visit for patients who withdrew from the study prior to week 52. Radiographs were scored using the van der Heijde-modified Sharp method [36, 37] for bone erosion, joint space narrowing (JSN) and total sharp score (TSS) independently by two readers who were well trained and competent to score radiographs in accordance with the method. The readers were blinded to the treatment group and chronological order of the films.

Clinical assessment

The Disease Activity Score on 28 joints (DAS28), clinical improvement in signs and symptoms of RA, tender joint count, swollen joint count, and modified health assessment questionnaire (MHAQ) [38] were assessed at baseline.

Laboratory examinations

Fasting blood samples and the second morning urine samples were obtained from all subjects at clinical visits. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured in the local clinical test laboratory of each investigation site.

To assess bone formation, we measured serum intact-osteocalcin (OC) using a two-site immunoradiometric assay (Mitsubishi Kagaku Iatron, Japan) and serum bone alkaline phosphatase (bone ALP) by an enzyme-linked immunosorbent analysis (ELISA; Quidel, San Diego, CA). Markers of bone resorption included urinary N-terminal crosslinked telopeptide of type I collagen (U-NTX-I), which was measured by an ELISA (Ostex Int, Seattle, WA), and urinary total deoxypyridinoline (U-DPD) and total pyridinoline (U-PYD), measured by a high-performance liquid chromatography (HPLC) assay. Markers of cartilage synthesis included the N-terminal propeptide of type IIA collagen (PIIANP; Linco, St. Louis, MO) and the C-terminal propeptide of type II collagen (PIICP; IBEX Diagnostics, Montreal, Canada). Cartilage degradation was assessed by the urinary excretion of the C-terminal

crosslinked telopeptide of type II collagen (CTX-II Cartilaps ELISA; NORDIC Biosciences, Herlev, Denmark). Synovial tissue metabolism was assessed by measuring the urinary excretion of glucosyl–galactosyl–pyridinoline (Glc–Gal–PYD) by HPLC, serum matrix metalloproteinase-3 (MMP-3) by ELISA (Daiichi Pure Chemical, Japan) and serum amyloid protein A (SAA) by a latex immunoassay (LIA; Eiken Chemical, Japan). Other measures included serum interleukin-6 (IL-6) using a chemiluminescent enzyme immunoassay (CLEIA) (Fujirebio Japan), RF by LIA (Mitsubishi Kagaku Iatron, Japan), and immunoglobulin G (IgG) by LIA (Eiken Chemical, Japan).

Statistical analysis

For analyzing the correlation between markers at baseline and at the 52-week radiological progression of joint damage, we normalized the markers by logarithmic transformation when needed. First, the markers were selected by Pearson correlation coefficient with TSS, erosion score, and JSN score ($|r| > 0.15$). Then, the predictive factors were selected based on the multivariate regression analysis using the backward elimination method, the forward selection method, and the best-subset selection procedure using Mallows' Cp- adjusted R^2.

The odds ratio of progression in TSS, bone erosion and JSN score according to the levels of these baseline factors were estimated by logistic regression analysis with a 95% confidence interval (95% CI). The progression of joint damage was defined as an increase of TSS of 0.5 or more at 52 weeks.

All statistical analyses were two-sided, and p values <0.05 were considered to be significant. All statistical analyses were carried out using SAS ver. 8.2, TS2MO (SAS Institute, Cary, NC).

Results

One hundred and forty-five patients were included in the intent to treatment (ITT) analyses. Demographics and baseline disease characteristics are shown in Tables 1 and 3. At baseline, the mean age and the disease duration were 53.1 and 2.4 years, respectively. Patients had very active disease, as indicated by a DAS28 score of 6.4 and CRP of 4.9 mg/dl at baseline. The kinds of DMARDs and immunosuppressants used for RA treatment during the study and the number of patients are shown in Table 2.

Bivariate linear correlation analyses showed that baseline values of U-PYD, the ratio U-PYD/DPD, U-CTX-II, U–Glc–Gal–PYD, TSS, erosion score, JSN score, age and BMI were associated significantly with the 1-year increase in all three radiological indices of joint damage, i.e. bone erosion score, JSN score and TSS (Table 3). The baseline levels of U-DPD, S-PIIANP, triglyceride, ferritin also had a significant association with one or two variables among these three radiographic progression parameters (Table 3). None of the clinical indices of disease activity nor the biological parameters of inflammation were associated significantly with radiological progression. In the

Table 1 Baseline demographics, clinical and laboratory characteristics of the patient cohort

Baseline demographics, clinical and laboratory characteristics	Values
Number of patients	145
Age, years (mean)	53.1 ± 12.5
Female, *n* (%)	119 (82.1)
BMI (kg/m^2)	21.8 ± 3.0
RA duration (years)	2.4 ± 1.3
Number of previous DMARDs	2.8
Tender joint count	14.4 ± 7.5
Swollen joint count	11.8 ± 5.8
CRP (mg/dl)	4.9 ± 2.9
DAS28	6.4 ± 0.9
Radiological total Sharp score	30.6 ± 42.0
Radiological bone erosion score	13.9 ± 21.7
Radiological joint space narrowing (JSN) score	16.7 ± 21.8

Values are given as the mean ± standard deviation, unless otherwise indicated

RA Rheumatoid arthritis, *DAS28* Disease Activity Score based on 28 joint counts, *CRP* C-reactive protein, *BMI* body mass index, *DMARDs* disease-modifying anti-rheumatic drugs

Table 2 Number of patients using concomitant drugs related to rheumatoid arthritis during the study

Variables	Number of patients[a]
Corticosteroids	145 (100%)
Methotrexate	123 (84.8%)
Mizoribine	11 (7.6%)
Azathioprine	7 (4.8%)
Ciclosporin	5 (3.4%)
Tacrolimus hydrate	3 (2.1%)
Sulfasalazine	60 (41.4%)
Bucillamine	33 (22.8%)
Sodium aurothiomalate	4 (2.8%)
D-Penicillamine	11 (7.6%)
Actarit	6 (4.1%)
Lobenzarit disodium	2 (1.4%)
Cyclophosphamide	2 (1.4%)
Minocycline hydrochloride	2 (1.4%)

[a] Values are given as the number of patients taking a drug; patients can take more than one drug

Table 3 Baseline patient measurements and Pearson correlation coefficient between the levels of candidate factors at baseline and the changes in radiographic score at week 52

Variables	Levels at baseline (mean ± SD)	*r* value between baseline levels and radiological progression at week 52		
		Total sharp score	Bone erosion score	Joint space narrowing (JSN) score
Bone markers				
Intact-osteocalcin (ng/ml)	5.1 ± 2.1	NS	NS	NS
Bone alkaline phosphatase (U/l)	21.5 ± 6.5	NS	NS	NS
S-NTX-I (nmol BCE/l)	15.8 ± 4.8	NS	NS	NS
U-NTX-I (nmol BCE/mmol creatinine)	62.6 ± 31.9	NS	NS	NS
U-DPD (μmol/mol creatinine)	8 ± 4	0.185*	NS	0.187*
Bone or cartilage markers				
U-PYD (μmol/mol creatinine)	55 ± 37	0.278**	0.253**	0.274**
U-PYD/DPD	7.2 ± 1.8	0.190*	0.180*	0.178*
Cartilage markers				
S-PIIANP (ng/ml)	459.8 ± 210.9	NS	−0.188*	NS
S-PIICP (ng/ml)	819.1 ± 311.6	NS	NS	NS
U-CTX-II (ng/mmol creatinine)	902.5 ± 919.2	0.356***	0.321***	0.356***
Radiographic scores				
Total Sharp score	16.7 ± 21.8	0.323***	0.303***	0.307***
Erosion score	30.6 ± 42.0	0.313***	0.308***	0.282**
Joint space narrowing score	13.9 ± 21.7	0.323***	0.291***	0.322***
Symptoms or functions				
DAS28	6.4 ± 0.9	NS	NS	NS
Objective signs				
Tender joint count	14.4 ± 7.5	NS	NS	NS
Swollen joint count	11.8 ± 5.8	NS	NS	NS
Patients reported functional assessment				
MHAQ	0.90 ± 0.58	NS	NS	NS
Inflammation markers				
CRP (mg/dl)	4.9 ± 2.9	NS	NS	NS
ESR (mm/h)	71 ± 25	NS	NS	NS
MMP-3 (ng/ml)	456.5 ± 347.5	NS	NS	NS
SAA (μg/ml)	347 ± 307	NS	NS	NS
Fibrinogen (mg/dl)	490 ± 96	NS	NS	NS
Interleukin-6 (pg/ml)	60.2 ± 64.9	NS	NS	NS
Synovium degradation marker				
U-Glc–Gal–PYD (nmol/mmol creatine)	11.6 ± 9.3	0.255**	0.238**	0.245**
Hematological parameters				
WBC (/μl)	8,923 ± 2,430	NS	NS	NS
RBC (10^4/μl)	397 ± 38	NS	NS	NS
Hemoglobin (g/dl)	11.3 ± 1.4	NS	NS	NS
Platelet (10^4/μl)	37.2 ± 10.1	NS	NS	NS
Lipid parameters				
Total cholesterol (mg/dl)	182 ± 33	NS	NS	NS
HDL cholesterol (mg/dl)	56 ± 14	NS	NS	NS
LDL cholesterol (mg/dl)	108 ± 27	NS	NS	NS
Triglyceride (mg/dl)	90 ± 35	−0.187*	−0.193*	NS
Other biomarkers				
RF (IU/ml)	247 ± 452	NS	NS	NS

Table 3 continued

Variables	Levels at baseline (mean ± SD)	*r* value between baseline levels and radiological progression at week 52		
		Total sharp score	Bone erosion score	Joint space narrowing (JSN) score
IgG (mg/dl)	1,697 ± 492	NS	NS	NS
Albumin (g/dl)	3.7 ± 0.3	NS	NS	NS
Ferritin (ng/ml)	105 ± 116	NS	−0.182*	NS
Age	53.1 ± 12.5	−0.259**	−0.278**	−0.205*
Gender (M:F)	26:119	NS	NS	NS
Duration of disease	2.4 ± 1.3	NS	NS	NS
Anthropometric factor				
BMI (kg/m^2)	21.8 ± 3.0	−0.298***	−0.257**	−0.311***

NS not significant, *S-NTX* Serum type I collagen cross-linked N-telopeptides, *U-NTX* urinary type I collagen cross-linked N-telopeptides, *U-DPD* urinary deoxypyridinoline, *U-PYD* urinary pyridinoline, *S-PIIANP* serum N-terminal propeptide of type IIA collagen, *S-PIICP* serum C-terminal propeptide of type II collagen, *U-CTX-II* urinary C-terminal telopeptide of type II collagen, *MHAQ* modified health assessment questionnaire, *ESR* erythrocyte sedimentation rate, *MMP-3* matrix metalloproteinase-3, *SAA* serum amyloid protein A, *U-Glc–Gal–PYD* urinary glucosyl–galactosyl–pyridinoline, *IgG* immunoglobulin G, *WBC* white blood cell, *RBC* red blood cell, *HDL cholesterol* high-density lipoprotein cholesterol, *LDL cholesterol* low-density lipoprotein cholesterol

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 4 Multivariate regression analysis relating JSN U-CTX-II, U-PYD/DPD, or BMI to changes in the radiographic scores at 52 weeks

Baseline predictor	Parameter estimate		*p* value
Total Sharp score progression			
JSN	4.88		0.04
PYD/DPD	20.81		0.02
CTX-II	9.41		<0.01
BMI	−0.92		<0.01
R^2		0.24	<0.001
Bone erosion progression			
PYD/DPD	11.20		0.04
CTX-II	5.58		<0.01
BMI	−0.48		0.02
R^2		0.17	<0.001
Joint space narrowing progression			
JSN	2.37		0.04
PYD/DPD	9.62		0.02
CTX-II	4.56		<0.01
BMI	−0.46		<0.01
R^2		0.25	<0.001

JSN Joint space narrowing, *PYD/DPD* logarithmic transformed urinary pyridinoline/deoxypyridinoline ratio, *CTX-II* logarithmic transformed urinary C-terminal telopeptide of type II collagen

multivariate analyses, increased levels of U-CTX-II, an increased U-PYD/DPD ratio and decreased BMI were the only independent predictors of the progression of bone erosion (Table 4). Together, these three variables explained 17% of the interindividual variance in the progression of bone erosion. For the progression of JSN and TTS, baseline JSN was also an independent predictor in addition to U-CTX-II, the U-PYD/DPD ratio and BMI (Table 4).

Logistic regression analysis after the categorization of the four predictive variables with the cut-off value of 500 ng/mmol/creatinine in U-CTX-II, median level for the U-PYD/DPD ratio, two cut-off values of 18.5 and 25 kg/m^2, respectively, in BMI and a 0 or >0 score in JSN score at baseline showed that the odds ratio for a yearly increase of TSS >0.5 was 2.6- to 9.9-fold higher risk in the high-risk group than in patients with low risk levels (Fig. 1a); the respective figures for progression in erosion score and for progression in JSN were 2.8–4.8 and 1.8–20.0, respectively (Fig. 1b, c). Baseline levels in the categorized groups are shown in Table 5.

Discussion

Based on our analysis of a panel of several demographical, clinical and laboratory parameters of disease activity, we found that increased urinary CTX-II, a high PYD/DPD ratio and low BMI were independent predictors of radiological progression in bone erosion and TTS in patients with RA receiving conventional DMARDs and that baseline JSN was also an independent predictor of radiological progression in JSN and TTS. These results suggest that these factors should be useful in identifying patients at high risk.

The bivariate analyses revealed that the baseline levels of U-PYD, the U-PYD/DPD ratio, U-CTX-II, TSS, erosion score, JSN score, U-Glc–Gal–PYD, age and BMI were

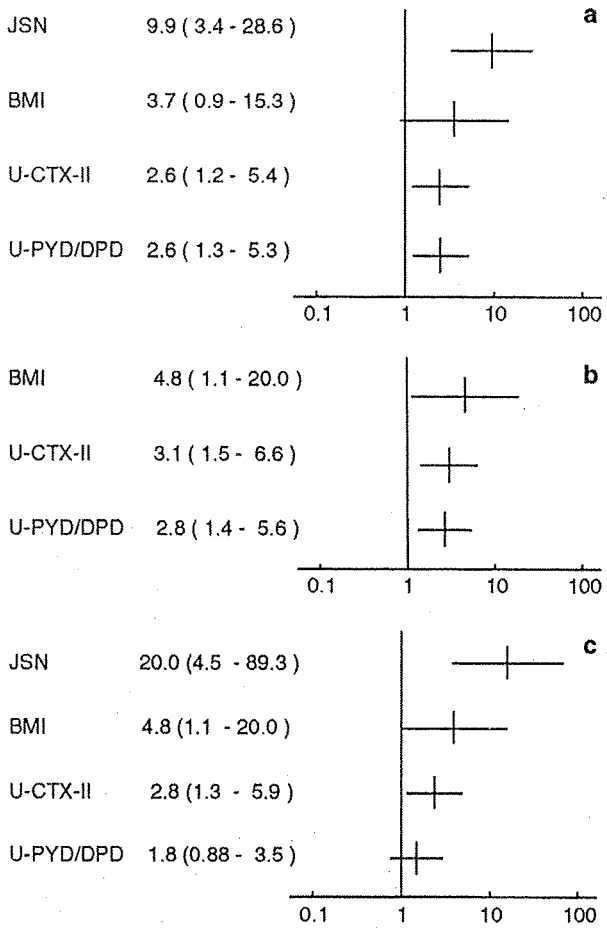

Fig. 1 Odds ratio (95% confidence interval) of radiological progression associated with high baseline joint space narrowing (*JSN*), high urinary C-terminal telopeptide of type II collagen (*U-CTXII*), high urinary total pyridinoline/total deoxypyridinoline (*U-PYD/DPD*), or low body mass index (*BMI*). Progression of joint damage over 1 year was defined as an increase >0.5 U of the total Sharp score (**a**), bone erosion (**b**) or JSN (**c**)

Table 5 Baseline levels in the categorized groups

Variables	Cut-off value	*n*	Mean of baseline value ± SD
JSN	0	30	0
	0<	115	21.1 ± 22.5
U-CTX-II (ng/mmol/creatinine)	<500	53	327.2 ± 104.6
	500≤	88	1,249.0 ± 1,014.9
U-PYD/DPD	<median (6.8)	72	5.8 ± 0.7
	Median (6.8)≤	73	8.6 ± 1.4
BMI (kg/m^2)	<18.5	20	17.5 ± 1.2
	18.5≤, <25	102	21.5 ± 1.6
	25≤	21	27.1 ± 1.7

significantly associated with the 1-year increase in all three indices of TSS, erosion score and JSN score and that the baseline levels of U-DPD, S-PIIANP, triglycerides and ferritin were significantly associated with one or two variables among these three radiographic progression parameters. However, there was no significant association with radiographic progression in the baseline levels of inflammation markers, MMP-3, hematological parameters, patients-reported functional assessments, such as MHAQ, and objective symptomatic scores. Although several previous studies showed that MMP-3 was predictive of radiological progression [22, 29, 39, 40] in RA, our data and those of Cunnane et al. [41] failed to reveal a significant association. Circulating MMP-3 levels have been reported to be significantly decreased after treatment with methotrexate or sulfasalazine or both together [29, 41–44]. These findings suggest that levels of MMP-3 are dependent on the type, duration and intensity of the pharmacotherapy. It is thus possible that differences in the therapeutic regimen between studies may explain some of the inconsistencies in the relation of MMP-3 to progression. Additional factors may include differences in disease duration and activity and variation in assay characteristics, which are not standardized between studies. Consistent with the results of a recent study [29], we confirmed that patient-reported functional assessments and clinical symptomatic indices were not useful in predicting radiological progression.

Inflammation markers, such as CRP and ESR, have been regarded as useful predictors of joint damage in RA. However, our study confirmed the recent findings of Young-Min [29], showing that when novel and more specific markers of joint tissue metabolism were included in the model, these unspecific laboratory tests were no longer predictive. Among these novel tissue turnover markers, the strongest and most consistent association with progression was observed for urinary CTX-II, a biochemical marker of cartilage degradation, a finding consistent with several previous studies involving patients with early RA receiving MTX or etanercept [19], very early RA receiving the COBRA combination therapy or sulfasalazine alone [45] or late RA treated with conventional DMARDs [29]. Taken together, the results from these previous studies and the current one suggest that urinary CTX-II is predictive of radiological progression across patient populations and independent of the type of therapy. We also found that urinary-Glc–Gal–PYD, a specific biochemical marker of synovial tissue metabolism, was associated significantly with radiographical progression in bivariate analysis. This result was consistent with that of a previous study [19] of early RA patients receiving methotrexate or etanercept. However, urinary-Glc–Gal–PYD did not remain in the final panel of predictors after multivariate analysis, confirming