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Clinical Importance of Body Composition in Improving Bone Mineral Density of Femoral Neck After Denosumab Therapy in Patients With Rheumatoid Arthritis or Collagen Diseases

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Abstract. *Background/Aim: To investigate factors associated with increased bone mineral density (BMD) of the neck of femur in rheumatoid arthritis or collagen diseases receiving denosumab, focusing on body composition calculated by bioelectrical impedance analysis (n=90, 78 females). Patients and Methods: We defined Δfemur as BMD (12 months minus baseline), using dual-energy X-ray absorptiometry after denosumab therapy. Factors associated with Δfemur were retrospectively investigated. Results: Low skeletal muscle index (SMI) was observed in 6 males and 32 females. There was a significant difference in phase angle (PhA) of the left leg (LL) between the Δfemur ≥0 (n=70) and Δfemur <0 (n=20) groups (p=0.040) but not in SMI (p=0.310). Multiple regression analysis indicated that PhA of LL was significantly related to Δfemur (p=0.0398). Conclusion: PhA appears to be a clinically significant indicator of improvement of Δfemur in patients receiving denosumab.*

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Denosumab, a fully human monoclonal antibody against receptor activator of nuclear factor-ϰB ligand (RANKL), inhibits osteoclastogenesis and activation by blocking the binding of RANKL to RANK, thereby reducing bone resorption, and increasing bone density (1-5). In Japan, denosumab has been covered by insurance as a new osteoporosis treatment since 2013, and its indications and usage are currently "osteoporosis" or "inhibition of the progression of bone erosion associated with rheumatoid arthritis (RA)". According to a Japanese phase III confirmatory clinical trial regarding the impact of denosumab on improvement in bone mineral density (BMD) in patients with primary osteoporosis, change ratios of BMD at 12 months after denosumab therapy were +6.6% in the lumbar spine and +2.8% in the neck of femur (6). These results indicate a large difference in improvement ratio between the lumbar spine and neck of femur. In clinical practice, we sometimes experience a patient treated with denosumab in whom BMD increases in the lumbar spine but shows no change in the neck of femur. Osteoporosis in the neck of femur is related to hip fractures and affects the patient's activities of daily life (7). Thus, at the bedside, it is important to find predictors associated with change in BMD of the femur (Δfemur).

Sarcopenia is characterized by progressive loss of skeletal mass and muscle strength and/or physical activity decline and is an essential component of the physical frailty syndrome (8, 9). Numerous studies have reported that osteoporosis and sarcopenia are closely related, and that both are attended by serious socioeconomic burdens (10-12). As sarcopenia and osteoporosis share many common factors, including age-related decreases in sex hormones and protein anabolic

hormones, vitamin D deficiency, and decreases in mechanical loading, these two pathological conditions appear profoundly linked. Osteoporosis is common in sarcopenic patients (*i.e.*, osteosarcopenia) and is associated with gait disturbance and loss of balance (13). Sarcopenia leads to loss of muscle mass and strength, leading to falls and fractures, and further to bone loss and loss of bone strength, which can then lead to an elevated risk of osteoporotic fragility fractures (7, 13-15). A relationship is postulated between sarcopenia and the ratio of BMD change in the neck of femur.

The use of data obtained from bioelectrical impedance analysis (BIA) has attracted much attention as an alternative to conventional error-prone calculation of body composition in diseases (16). BIA is widely used to estimate skeletal muscle mass and evaluate sarcopenia (7, 14). Skeletal muscle index (SMI) is calculated as the ratio of skeletal muscle mass to body surface area, with low SMI (<7.0 kg/m^2 in men, <5.7 kg/m^2 in women) indicating sarcopenia (7, 14). In addition, BIA offers a practical approach to estimation of muscle mass, fluid status, and nutritional status by evaluating whole-body cell membrane quality and depicting fluid distribution in the human body (16). Phase angle (PhA) is calculated directly from BIA measurements without an estimating equation and reflects the physiological function of cells. The extracellular water (ECW)-to-total body water (TBW) ratio (ECW/TBW) is a measure of the severity of pericellular edema (17).

However, to the best of our knowledge, no study has examined the relevance of body composition to the improvement in BMD among patients with RA or collagen disease who are undergoing denosumab therapy. Clarification of these issues appears clinically meaningful. The aim of this study was to investigate factors associated with improvement in BMD, especially in the neck of femur, among patients with RA or collagen diseases receiving denosumab therapy, with a particular focus on body composition analysis.

Patients and Methods

Patients. We carefully reviewed the medical records of 151 patients with RA or collagen diseases who visited Osaka University Hospital for consultation between July 2013 and September 2018. BIA or dual-energy X-ray absorptiometry (DEXA) data were missing for 61 patients, who were therefore excluded from analysis. Thus, a total of 90 patients were analyzed in this study. All analyzed patients received denosumab therapy (60 mg, subcutaneously) every 6 months based on accepted guidelines. DEXA was performed at baseline and at 6 and 12 months to calculate BMD. BIA was performed at 12 months after denosumab therapy. For all patients, appropriate management of underlying diseases was undertaken by expert physicians. An activated vitamin D preparation was given to all patients except for one with hypercalcinemia. In most cases, maintenance corticosteroid dose was not changed during the observation period.

Study factors. Change in BMD in the lumbar spine at 12 months (Δlumbar) was defined as the BMD in the lumbar spine (L2-4) at 12 months minus that at baseline; and change in BMD at 12 months at the neck of femur (Δfemur) was defined as BMD at the neck of femur at 12 months minus that at baseline. The following factors associated with Δlumbar and Δfemur were examined retrospectively to determine the relationships among SMI, ECW/TBW and PhA: age, sex, body mass index (BMI), serum albumin, estimated glomerular filtration rate (eGFR), alanine aminotransferase (ALT), C reactive protein (CRP), tartrate-resistant Acid Phosphatase 5b (TRACP5b), urine type 1 collagen cross-linked N-telopeptid (NTx), PhA, ECW/TBW, SMI, corticosteroid maintenance dose calculated as prednisolone (PSL), and percentage of bisphosphonate usage before denosumab therapy. BMD of the left neck of femur was measured by DEXA at Osaka University Hospital. Body composition data was directly estimated by InBody770 (Biospace, Seoul, Republic of Korea). The reference ranges for low SMI (defined as appendicular muscle mass divided by height squared (kg/m^2)] were <7.0 kg/m^2 for men and <5.7 kg/m^2 for women, in BIA based on the current Asian guidelines (7).

We obtained ethical approval from the ethics committee of Osaka University Hospital (No. 14269-4) and the protocol of our study strictly adhered to all regulations of the Declaration of Helsinki. An opt-out approach was employed due to the retrospective nature of the study.

Statistical analysis. Descriptive statistics are presented as the mean±standard deviation (SD) or as numbers and percentages. Pearson correlation, unpaired *t*-test, and paired *t*-test were used to assess continuous variables between and within groups, whereas Fisher's exact test was used to compare the percentages of categorical variables between groups. Multiple regression analysis was performed to identify factors associated with Δfemur. We considered factors with a *p*-value less than 0.05 as statistically significant. All analysis was performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Changes in BMD after denosumab therapy. Table I lists the baseline background characteristics in the study cohort (n=90, 78 females). Underlying disease included RA (n=26), systemic lupus erythematosus (SLE) (n=25), progressive systemic sclerosis (n=9), Behçet's disease (n=6), mixed connective tissue disease (MCTD) (n=4), dermatomyositis (n=3), polymyalgia rheumatica (n=3), and others (n=14). Mean BMD in the lumbar spine at baseline, 6 months, and 12 months was 0.62±0.12 g/cm^2, 0.63±0.12 g/cm^2, and 0.64±0.13 g/cm^2, respectively (baseline *vs.* 12 months, $p<0.0001$, *paired-t test*) (data not shown). Mean BMD values in the neck of femur at baseline, 6 months, and 12 months were 0.89±0.17 g/cm^2, 0.91±0.17 g/cm^2, and 0.93±0.18 g/cm^2 (baseline *vs.* 12 months, $p<0.0001$, *paired-t test*) (data not shown). Δlumbar was <0 in 12 patients (13.3%), whereas Δfemur was <0 in 20 patients (22.2%), indicating the difference in response to treatment with denosumab according to evaluation site (Figure 1). The present improvement ratio between the lumbar spine and neck of femur was the same as

Table I. *Disease characteristics and therapeutic background according to Δfemur.*

Disease and therapy	Overall (n=90)	Δfemur ≥0 (n=70)	Δfemur <0 (n=20)	*p*-Value
RA (%)	26 (28.8)	21 (30.0)	5 (25.0)	0.784
SLE (%)	25 (27.7)	22 (31.4)	3 (15.0)	0.171
SSc (%)	9 (10.0)	7 (10.0)	2 (10.0)	1.000
Behçet's disease (%)	6 (6.7%)	4 (5.7)	2 (10.0)	0.611
MCTD (%)	4 (4.4%)	1 (1.4)	3 (15.0)	0.033
DM (%)	3 (3.3%)	2 (2.8)	1 (5.0)	0.534
PMR (%)	3 (3.3%)	2 (2.8)	1 (5.0)	0.534
IDDM (%)	1 (1.1%)	0	1 (5.0)	0.222
AGA (%)	1 (1.1%)	0	1 (5.0)	0.222
Alopecia totalis (%)	1 (1.1%)	0	1 (5.0)	0.222
Others (%)	11 (12.2%)	11 (15.7)	0	0.114
PSL (mg/day)	4.41 (3.48)	4.12 (3.47)	5.42 (3.43)	0.141
Active Vit-D (%)	89 (98.8%)	69 (98.5)	20 (100)	1.000
Bisphosphonate (%)	58 (64.4%)	45 (64.2)	13 (65.0)	1.000

Data are shown as number and percentage except for PSL. PSL is shown as mean value (standard deviation). RA: Rheumatoid arthritis; SLE: systemic lupus erythematous; SSc: systemic sclerosis; MCTD: mixed connective tissue disease; DM: dermatomyositis; PMR: polymyalgia rheumatica; IDDM: insulin dependent diabetes mellitus; AGA: allergic granulomatous angiitis; PSL: prednisolone.

that reported previously (6). Response differed in individual patients after denosumab therapy. Figure 2 shows the results for two female SLE patients of a similar age: one aged 45 years who received PSL 4 mg/day, activated vitamin D, and minodronic acid hydrate 50 mg; and another aged 43 years who received PSL 12.5 mg/day, activated vitamin D, and minodronic acid hydrate 50 mg. The effect of denosumab on BMD values in the neck of femur was very different between these patients. Accordingly, we investigated the clinical factors related to the effect of denosumab on BMD values in the neck of femur.

Relationship between patient background and Δfemur. Values of Δfemur were <0 in 20 patients and ≥0 in 70 patients. Significant difference was reached only between Δfemur <0 (n=3) and Δfemur ≥0 (n=1) in patients with MCTD (p=0.033, *Fisher's exact test*). However, the ratio between Δfemur ≥0 and Δfemur <0 was almost the same in other diseases. At the maintenance dose of corticosteroid calculated as PSL, values tended to be higher in the Δfemur <0 group than in the Δfemur ≥0 group, but the difference was not statistically significant (Δfemur ≥0, 4.12±3.47 *vs*. Δfemur <0, 5.42±3.43 mg/day; p=0.141, unpaired t-test). The percentage usages of activated vitamin D and bisphosphonate were similar.

Table II shows the values of clinical data according to classis factors of osteoporosis. Mean age tended to be higher in the Δfemur <0 group than in the Δfemur ≥0 group, but the difference was not statistically significant (Δfemur ≥0, 53.91±13.77 years *vs*. Δfemur <0, 56.70±14.58 years; p=0.493, unpaired t-test). There was no statistically significant difference between the two groups in terms of sex, BMI, serum albumin, eGFR, AST, ALT, or CRP. Blood levels of TRACP5b and urinary levels of NTx, which are markers of bone metabolism, were similar between the groups (TRACP5b: Δfemur ≥0, 160.07±99.88 *vs*. Δfemur <0, 154.37±79.22 mU/dl; p=0.819. NTx: Δfemur ≥0, 22.08±14.70 *vs*. Δfemur <0, 19.44±11.27 nmolBCE/nmol/CRE; p=0.482, unpaired t-test).

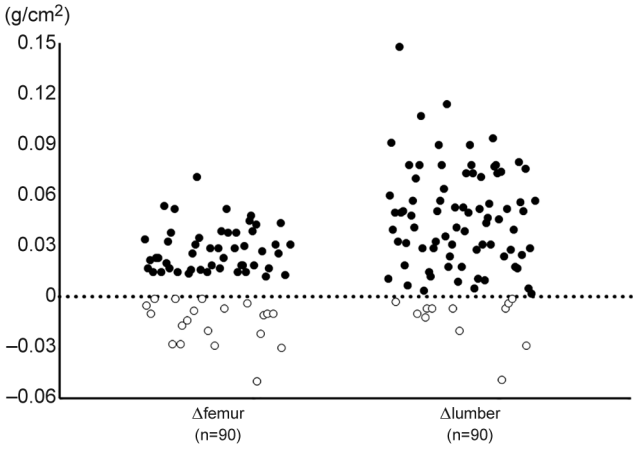

Figure 1. *Comparison of bone mineral density values in the lumbar spine (Δlumbar) and neck of femur (Δfemur) between baseline and at 12 months after denosumab therapy (n=90) as assessed by dual-energy X-ray absorptiometry. Δlumbar was defined as BMD at 12 months in the lumbar spine (L2-4) minus BMD at baseline in the lumbar spine (L2-4), and Δfemur was defined as BMD at 12 months in the neck of femur minus BMD at baseline in the neck of femur.*

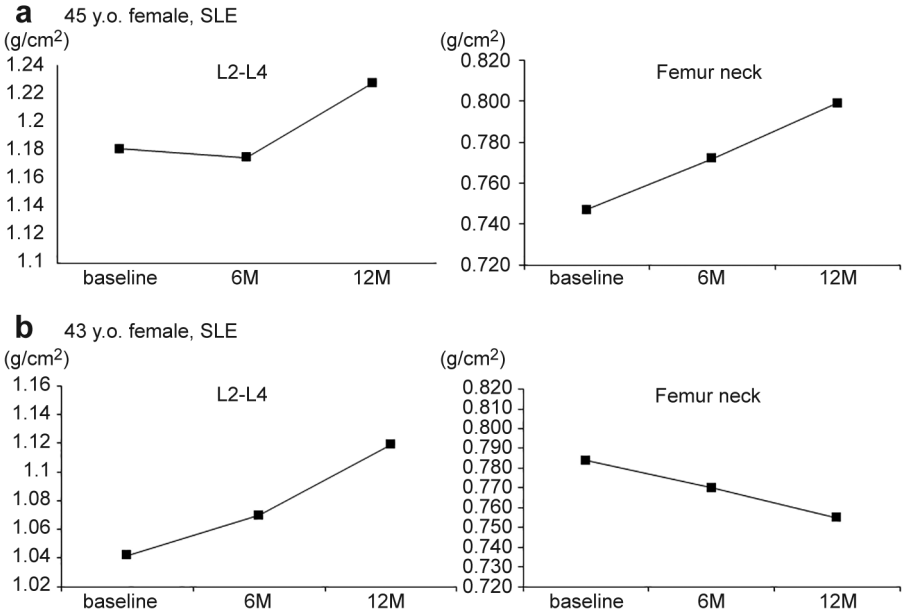

Figure 2. *Representative data of changes of bone mineral density (BMD) values in the lumbar spine (L2-4) and neck of femur during one year of denosumab therapy, as assessed by dual-energy X-ray absorptiometry. (A) A 45-year-old female patient with systematic lupus erythematous (SLE). BMD values for both the lumbar spine (L2-4, left) and neck of femur (right) were highest at 12 months. This patient received 4 mg/day of prednisolone. (B) A 43-year-old female patient with SLE. BMD values showed a steady increase in the lumbar spine (L2-4) but those in the neck of femur decreased steadily over the 12-month period. This patient received 12.5 mg/day of prednisolone.*

Table II. *Clinical data according to Δfemur level.*

Variables	Overall (n=90)	Δfemur ≥0 (n=70)	Δfemur <0 (n=20)	*p*-Value
Age (year)	54.53 (13.91)	53.91 (13.77)	56.70 (14.58)	0.433
Height (cm)	158.04 (9.22)	158.00 (10.08)	158.21 (5.43)	0.930
Body weight (kg)	53.15 (9.67)	53.06 (9.80)	53.46 (9.43)	0.870
BMI (kg/m^2)	21.23 (3.06)	21.20 (3.00)	21.32 (3.34)	0.883
Male/female (%)	11/79 (12.2/87.8)	9/61 (12.9/87.1)	2/18 (10.0/90.0)	1.000
Albumin (g/dl)	4.13 (0.30)	4.15 (0.29)	4.06 (0.32)	0.300
CRP (mg/dl)	0.050 (0.13)	0.050 (0.11)	0.055 (0.17)	0.975
Creatinine (mg/dl)	0.66 (0.16)	0.66 (0.16)	0.65 (0.15)	0.823
eGFR (ml/min/1.73 m^2)	78.17 (16.42)	78.21 (16.21)	78.03 (17.53)	0.965
AST (IU/l)	23.03 (7.86)	23.41 (8.36)	21.70 (5.72)	0.393
ALT (IU/l)	20.17 (12.93)	20.29 (13.46)	19.75 (11.18)	0.871
γ-GTP (IU/l)	32.35 (23.52)	31.33 (21.37)	36.11 (30.56)	0.435
TRACP5b (mU/dl)	158.83 (95.34)	160.07 (99.88)	154.37 (79.22)	0.819
Urinary levels of NTX (nmolBCE/mmol×CRE)	21.51 (14.00)	22.08 (14.70)	19.44 (11.27)	0.482

Data are shown as mean value (standard deviation) except for CRP. CRP is shown as median value (interquartile range). BMI: Body mass index; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GTP: γ-Glutamyl transpeptidase; TRACP5b: tartrate-resistant acid phosphatase 5b; NTx: type 1 collagen cross-linked N-telopeptide.

Relationship between body composition data and Δfemur. Table III shows the relationship between body composition characteristics and increased ratio of BMD in the neck of femur. SMI tended to be lower in the Δfemur <0 group than in the Δfemur ≥0 group, but the difference was not statistically significant (Δfemur ≥0; 5.93±0.91 *vs.* Δfemur <0, 5.71±0.60 kg/m^2; p=0.310, unpaired t-test). In males, low SMI (<7.0 kg/m^2) was observed in 6 patients (Δfemur

Table III. *Characteristic of body composition data according to Δfemur level.*

Parameters	Overall (n=90)	Δfemur ≥0 (n=70)	Δfemur <0 (n=20)	*p*-Value
Skeletal muscle mass (kg)	19.70 (4.21)	19.99 (4.52)	18.69 (2.73)	0.227
SMI (kg/m^2)	5.88 (0.85)	5.93 (0.91)	5.71 (0.60)	0.310
Low SMI <7.0 (kg/m^2), male (%)	6 (6.7)	4 (5.7)	2 (10.0)	0.611
Low SMI <5.7 (kg/m^2), female (%)	32 (35.6)	27 (38.6)	5 (25.0)	0.302
Body fat mass (kg)	16.18 (5.88)	16.27 (6.05)	15.88 (5.39)	0.799
Percent body fat (PBF) (%)	29.73 (7.53)	29.60 (7.75)	30.17 (6.86)	0.768
ECW/TBW whole body	0.393 (0.009)	0.392 (0.008)	0.396 (0.011)	0.055
ECW/TBW whole body ≥0.4	32 (35.5)	25 (35.7)	7 (35.0)	1.000
ECW/TBW of RA	0.382 (0.005)	0.382 (0.004)	0.382 (0.005)	0.975
ECW/TBW of LA	0.382 (0.005)	0.382 (0.005)	0.383 (0.005)	0.416
ECW/TBW of trunk	0.393 (0.009)	0.392 (0.008)	0.397 (0.011)	0.032
ECW/TBW of RL	0.394 (0.010)	0.393 (0.009)	0.397 (0.012)	0.084
ECW/TBW of LL	0.396 (0.010)	0.394 (0.009)	0.399 (0.011)	0.081
Phase angle whole body	4.40 (0.71)	4.45 (0.70)	4.22 (0.72)	0.195
Phase angle of RA	4.21 (0.65)	4.22 (0.67)	4.16 (0.62)	0.723
Phase angle of LA	4.03 (0.64)	4.07 (0.66)	3.91 (0.59)	0.316
Phase angle of trunk	7.03 (1.00)	7.11 (1.03)	6.76 (0.85)	0.157
Phase angle of RL	4.43 (0.88)	4.52 (0.84)	4.08 (0.96)	0.049
Phase angle of LL	4.42 (0.89)	4.52 (0.87)	4.07 (0.89)	0.040

Data are shown as mean value (standard deviation). SMI: Skeletal muscle index; ECW: extracellular water; TBW: total body water; RA: right arm; LA: left arm; RL; right leg; LL: left leg.

≥0, 4 (44.4%) *vs.* Δfemur <0, 2 (100); p=0.611, Fisher's exact test); whereas in females, low SMI (<5.7 kg/m^2) was observed in 32 patients (Δfemur ≥0, 27 (44.2%) *vs.* Δfemur <0, 5 (27.7%); p=0.302, Fisher's exact test).

In addition, among the various data acquired by the body composition analyzer, ECW/TBW and PhA are representative data. ECW/TBW of the left leg (LL) 0.4, indicating a moderate-to-severe edematous state, was observed in 32 patients (35.5%) [Δfemur ≥0, 25 (35.7%) *vs.* Δfemur <0, 7 (35.0%); p=1.0, Fisher's exact test]. ECW/TBW whole body values tended to be lower in the Δfemur <0 group than in the Δfemur ≥0 group (Δfemur ≥0, 0.392±0.008 *vs.* Δfemur <0, 0.396±0.011; p=0.055, unpaired *t*-test). Mean PhA whole body was 4.40±0.71. Statistically significant difference was found for PhA of the right leg (RL) (Δfemur ≥0, 4.52±0.84 *vs.* Δfemur <0, 4.08±0.96˚; p=0.049, unpaired *t*-test) and PhA of LL (Δfemur ≥0, 4.52±0.87 *vs.* Δfemur <0, 4.07±0.89˚, p=0.040, unpaired *t*-test).

Multiple regression analysis of ECW/TBW and PhA for $\Delta 12_{femur}$. ECW/TBW of the trunk was significantly different between the two groups. However, because trunk data are generally affected by diet as well as various clinical factors, we did not subject these data to multiple regression analysis. We evaluated the change in BMD in the left neck of femur using DEXA, and then performed multiple regression analysis of ECW/TBW and PhA of LL for Δfemur.

PhA of LL correlated very strongly and negatively with ECW/TBW of LL (r=–0.95, p<0.0001, Pearson correlation; data not shown). Thus, ECW/TBW of LL and PhA of LL or Δfemur were analyzed separately. Age, sex, BMI, PSL, and SMI were included in multiple regression analysis to adjust for other factors known to be associated with osteoporosis. In multiple regression analysis, association with Δfemur and PhA of LL (p=0.0398) but not ECW/TBW of LL (p=0.0578) were identified as significant factors (Table IV and Table V).

Discussion

In this study, we investigated the clinical factors associated with increases in BMD of the neck of femur at 12 months after denosumab therapy among patients with RA or collagen diseases. Our clinical results regarding the effect of denosumab on BMD values in the lumbar spine and the neck of femur were consistent with those reported previously (6). We found no relationship between Δ femur, and the classic factors related to osteoporosis; *i.e.*, age, sex, BMI, TRACP5b, and urinary level of NTx. Surprisingly, body composition data, especially ECW/TBW and PhA, might be predictors of efficacy of denosumab treatment in terms of BMD of the femoral neck. In our multiple regression analysis, PhA but not ECW/TBW of the left leg was an independent factor linked to Δ femur. The importance of body composition data to BMD, especially PhA, in patients with RA or collagen diseases receiving denosumab therapy

Table IV. *Multi-regression analysis for Δfemur BMD using ECW/TBW.*

Parameter	Estimated value	Standard error	t value	Pr >|t|	95% Confidence interval	
Intercept	0.233785709	0.12049168	1.94	0.0557	–0.005867397	0.473438815
Age	-9.54142E-05	0.00023087	–0.41	0.6805	–0.000554611	0.000363782
Sex	0.009014805	0.00805706	1.12	0.2664	–0.007010373	0.025039982
BMI	-0.000456146	0.00080104	–0.57	0.5706	–0.002049379	0.001137088
PSL	-0.000778072	0.00066734	–1.17	0.2470	–0.002105391	0.000549248
SMI	0.003804235	0.00364988	1.04	0.3003	–0.003455225	0.011063695
ECW/TBW of LL	-0.586044305	0.30464382	–1.92	0.0578	–1.191968601	0.019879992

BMD: Bone mineral density; ECW: Extracellular water; TBW: total body water; LL: left leg; BMI: body mass index; PSL: prednisolone; SMI: skeletal muscle index.

Table V. *Multi-regression analysis for Δfemur BMD using phase angle.*

Parameter	Estimated value	Standard error	t value	Pr >|t|	95% Confidence interval	
Intercept	–0.015180281	0.0311458	–0.49	0.6273	–0.077128026	0.046767465
Age	–0.000108659	0.00021949	–0.5	0.6219	–0.000545217	0.0003279
Sex	0.007079327	0.00815778	0.87	0.3880	–0.009146169	0.023304822
BMI	–0.000535034	0.00080172	–0.67	0.5064	–0.002129631	0.001059563
PSL	–0.000722871	0.00066842	–1.08	0.2826	–0.002052327	0.000606584
SMI	0.001637348	0.00389456	0.42	0.6753	–0.006108766	0.009383463
Phase angle of LL	0.007644879	0.00366062	2.09	0.0398	0.000364052	0.014925706

BMD: Bone mineral density; ECW: extracellular water; TBW: total body water; LL: left leg; BMI: body mass index; PSL: prednisolone; SMI: skeletal muscle index.

was therefore highlighted by our results. To the best of our knowledge, this is the first study to demonstrate an impact of body composition data on improvement of BMD at the femoral neck in patients with RA or collagen diseases undergoing denosumab therapy.

In the phase III clinical trial mentioned earlier, change ratios of BMD at 12 months after denosumab therapy were +6.6% in the lumbar spine and +2.8% in the neck of femur, which are substantially different (6). Similar findings were seen in our results; that is, several cases showed no increase in BMD at the neck of femur despite the increased BMD in the lumbar spine during denosumab therapy. In other words, there was an inferior response rate to denosumab therapy in the neck of femur compared with the lumbar spine. The neck of the femur is prone to fractures and consequently worsening of a patient's quality of life in the event of a fracture (7). Thus, predictors associated with Δfemur seem important in the clinical setting.

Skeletal muscle represents the largest organ in the human body, accounting for 40%-50% of body weight (18). Skeletal muscle mass is maintained at a constant level by the balance between protein catabolic and anabolic effects (18). The understanding of sarcopenia has been rapidly evolving in clinical and research fields, especially in the last few years, since the concept was proposed by Rosenberg in 1989 (19, 20). Primary sarcopenia is related to aging, whereas secondary sarcopenia is caused by the disease burden itself (8, 19). In patients with RA, longer disease duration has been associated with a decline in grip strength (21). A previous comparative study reported that sarcopenic obesity (*i.e.*, sarcopenia combined with obesity) was present in 6.5% of SLE and in 5.6% of RA women, but not in any controls, and that female patients with SLE or RA were more likely to show an abnormal body composition phenotype (22). In the present results, low SMI was observed in 38 patients (42.2%), although data for grip strength (indicating sarcopenia) were missing. In a large Japanese survey (n=4,811), the prevalence of sarcopenia was 7.5% (23). Clinicians should be aware of the high prevalence of muscle mass loss among patients with chronic inflammatory diseases. In contrast, BMD values of the lumbar spine and neck of femur at baseline in the low-SMI group were significantly lower than those in the group showing normal SMI, in agreement with previous reports (24). Miyakoshi *et al.* reported complication rates of sarcopenia stratified by BMD in 2,400 patients, and rates were 10.4%, 16.8%, and 20.4% in the normal lumbar BMD group, BMD loss group, and osteoporosis group, respectively (24). Indeed, sarcopenia may be closely linked to osteoporotic status, referred to as "osteosarcopenia". A previous meta-analysis reported that

lower BMI confers an elevated risk for any type of fracture that is independent of age and sex, but dependent on BMD (25). The Japan Society for the Study of Obesity defines a BMI of 22 kg/m^2 as the appropriate weight that is statistically the least susceptible to disease. Additional nutritional guidance may be required for patients who have RA or collagen diseases and low BMI. However, our multi-regression analysis using SMI and BMI showed that PhA was an independent factor related to Δfemur.

PhA can be noninvasively and easily obtained by BIA. PhA is the ratio of resistance (resistance inside and outside the cell, such as lipid components) to reactance (resistance specific to the cell membrane), expressed as an angle. PhA reflects the health of the cell membrane and structural stability of the cell (26). As a PhA of 0° indicates cell destruction, the lower the PhA, the worse the health and function of the cell (26). PhA decreases with age, and the decrease in PhA can be associated with sarcopenia (27, 28). Severe dietary restriction and malnutrition cause excessive production of free radicals, which damage cells (29), and the decrease in PhA reflects this damage. When cell membranes are weakened (*i.e.*, low PhA), long-term prognosis can be affected by accelerated aging and lowered immune function (30). As PhA reflects nutritional status and increases with improved nutritional status, this value can be a useful indicator to evaluate the effects of dietary therapy (29, 30). PhA is also reported to be a helpful predictor in patients receiving surgery or with advanced malignancies (31-34). PhA is considered a reliable marker of malnutrition, and the change in PhA was adopted as a primary endpoint in a recent randomized clinical trial of nutritional intervention for malnourished patients with advanced cancer (33). In addition, a very strong negative correlation (r=–0.95, p<0.0001, Pearson correlation; data not shown) was found between PhA and ECW/TBW in our data, and ECW/TBW thus offers an alternative marker to PhA. Considering these findings, appropriate nutritional intervention during denosumab therapy in patients with RA or collagen diseases may be essential for improving BMD, alongside appropriate medical therapies for underlying diseases. Long-standing chronic inflammatory status such as RA can cause an edematous state (*i.e.*, elevation of ECW/TBW) and malnutrition (*i.e.*, reduction in PhA). The normal range of PhA is reported as 4.87-9.17˚ (26, 35, 36). In the present data, including those of MCTD patients, mean PhA was 4.40±0.71° at baseline. Therefore, a considerable number of patients were in a malnourished state due to underlying disease burden. Our results indicate that edematous state and malnutrition are related to decreases in BMD of the neck of femur.

Several limitations associated with this study warrant mention. First, our study was a single-center, retrospective, observational study, and thus additional validation studies in independent cohorts are needed. Second, all analyzed patients were recruited from Japanese patients with RA or collagen diseases. Whether these results are applicable to RA or collagen disease patients from different ethnic backgrounds requires additional research. Third, various collagen diseases were included but the disease duration in each underlying disease was not included in our analysis, representing another source of bias. Finally, muscle strength as assessed by grip strength for the evaluation of sarcopenia was not used in this study. Our results should therefore be verified in future studies. Nevertheless, our results suggest that body composition data, especially PhA, could be useful for predicting improvements in BMD for patients on denosumab therapy.

In conclusion, we would like to emphasize the clinical significance of body composition on improvement of BMD at the neck of femur in patients with RA or collagen diseases who are receiving denosumab therapy. Earlier nutritional intervention in patients with malnutrition may be required to inhibit decreases in BMD.

Conflicts of Interest

Dr. Hagihara holds the position of Joint Research Chair in collaboration with Tsumura Co. Dr. Egashira is a member of the Joint Research group. Dr. Matsui has received research grants from Chugai and Asahi Kasei. The other Authors have no competing interests regarding this study.

Authors' Contributions

Dr. K.H. had full access to all data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. Concept and design: K.H. Acquisition, analysis, or interpretation of data: all Authors. Drafting of the manuscript: K.H., R.E., H.N., M.N. Statistical analysis: A.M. Administrative, technical, or material support: K.H. Final approval of manuscript: all Authors.

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References

1 Chiu YG and Ritchlin CT: Denosumab: targeting the RANKL pathway to treat rheumatoid arthritis. Expert Opin Biol Ther *17(1)*: 119-128, 2017. PMID: 27871200. DOI: 10.1080/14712598.2017.1263614

2 Etich J, Leßmeier L, Rehberg M, Sill H, Zaucke F, Netzer C and Semler O: Osteogenesis imperfecta-pathophysiology and therapeutic options. Mol Cell Pediatr *7(1)*: 9, 2020. PMID: 32797291. DOI: 10.1186/s40348-020-00101-9

3 Sinningen K, Tsourdi E, Rauner M, Rachner TD, Hamann C and Hofbauer LC: Skeletal and extraskeletal actions of denosumab.

Endocrine *42(1)*: 52-62, 2012. PMID: 22581255. DOI: 10.1007/s12020-012-9696-x

4 Bone HG, Bolognese MA, Yuen CK, Kendler DL, Miller PD, Yang YC, Grazette L, San Martin J and Gallagher JC: Effects of denosumab treatment and discontinuation on bone mineral density and bone turnover markers in postmenopausal women with low bone mass. J Clin Endocrinol Metab *96(4)*: 972-980, 2011. PMID: 21289258. DOI: 10.1210/jc.2010-1502

5 Bonnet N, Bourgoin L, Biver E, Douni E and Ferrari S: RANKL inhibition improves muscle strength and insulin sensitivity and restores bone mass. J Clin Invest *129(8)*: 3214-3223, 2019. PMID: 31120440. DOI: 10.1172/JCI125915

6 Nakamura T, Matsumoto T, Sugimoto T, Hosoi T, Miki T, Gorai I, Yoshikawa H, Tanaka Y, Tanaka S, Sone T, Nakano T, Ito M, Matsui S, Yoneda T, Takami H, Watanabe K, Osakabe T, Shiraki M and Fukunaga M: Clinical Trials Express: fracture risk reduction with denosumab in Japanese postmenopausal women and men with osteoporosis: denosumab fracture intervention randomized placebo controlled trial (DIRECT). J Clin Endocrinol Metab *99(7)*: 2599-2607, 2014. PMID: 24646104. DOI: 10.1210/jc.2013-4175

7 Oliveira A and Vaz C: The role of sarcopenia in the risk of osteoporotic hip fracture. Clin Rheumatol *34(10)*: 1673-1680, 2015. PMID: 25912213. DOI: 10.1007/s10067-015-2943-9

8 Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, Jang HC, Kang L, Kim M, Kim S, Kojima T, Kuzuya M, Lee JSW, Lee SY, Lee WJ, Lee Y, Liang CK, Lim JY, Lim WS, Peng LN, Sugimoto K, Tanaka T, Won CW, Yamada M, Zhang T, Akishita M and Arai H: Asian Working Group for Sarcopenia: 2019 Consensus update on sarcopenia diagnosis and treatment. J Am Med Dir Assoc *21(3)*: 300-307.e2, 2020. PMID: 32033882. DOI: 10.1016/j.jamda.2019.12.012

9 Nishikawa H, Shiraki M, Hiramatsu A, Moriya K, Hino K and Nishiguchi S: Japan Society of Hepatology guidelines for sarcopenia in liver disease (1st edition): Recommendation from the working group for creation of sarcopenia assessment criteria. Hepatol Res *46(10)*: 951-963, 2016. PMID: 27481650. DOI: 10.1111/hepr.12774

10 Edwards MH, Dennison EM, Aihie Sayer A, Fielding R and Cooper C: Osteoporosis and sarcopenia in older age. Bone *80*: 126-130, 2015. PMID: 25886902. DOI: 10.1016/j.bone.2015.04.016

11 Reginster JY, Beaudart C, Buckinx F and Bruyère O: Osteoporosis and sarcopenia: two diseases or one? Curr Opin Clin Nutr Metab Care *19(1)*: 31-36, 2016. PMID: 26418824. DOI: 10.1097/MCO.0000000000000230

12 Ji HM, Han J and Won YY: Sarcopenia and osteoporosis. Hip Pelvis *27(2)*: 72-76, 2015. PMID: 27536606. DOI: 10.5371/hp.2015.27.2.72

13 Waters DL, Hale L, Grant AM, Herbison P and Goulding A: Osteoporosis and gait and balance disturbances in older sarcopenic obese New Zealanders. Osteoporos Int *21(2)*: 351-357, 2010. PMID: 19436938. DOI: 10.1007/s00198-009-0947-5

14 Cederholm T, Cruz-Jentoft AJ and Maggi S: Sarcopenia and fragility fractures. Eur J Phys Rehabil Med *49(1)*: 111-117, 2013. PMID: 23575205.

15 Tanimoto Y, Watanabe M, Sun W, Sugiura Y, Hayashida I, Kusabiraki T and Tamaki J: Sarcopenia and falls in community-dwelling elderly subjects in Japan: Defining sarcopenia according to criteria of the European Working Group on Sarcopenia in Older People. Arch Gerontol Geriatr *59(2)*: 295-299, 2014. PMID: 24852668. DOI: 10.1016/j.archger.2014.04.016

16 Lee Y, Kwon O, Shin CS and Lee SM: Use of bioelectrical impedance analysis for the assessment of nutritional status in critically ill patients. Clin Nutr Res *4(1)*: 32-40, 2015. PMID: 25713790. DOI: 10.7762/cnr.2015.4.1.32

17 Nishikawa H, Yoh K, Enomoto H, Ishii N, Iwata Y, Nakano C, Takata R, Nishimura T, Aizawa N, Sakai Y, Ikeda N, Hasegawa K, Takashima T, Iijima H and Nishiguchi S: Extracellular water to total body water ratio in viral liver diseases: a study using bioimpedance analysis. Nutrients *10(8)*: 1072, 2018. PMID: 30103528. DOI: 10.3390/nu10081072

18 Wiedmer P, Jung T, Castro JP, Pomatto LCD, Sun PY, Davies KJA and Grune T: Sarcopenia - Molecular mechanisms and open questions. Ageing Res Rev *65*: 101200, 2021. PMID: 33130247. DOI: 10.1016/j.arr.2020.101200

19 Rosenberg I: Summary comments. The American Journal of Clinical Nutrition *50(5)*: 1231-1233, 2018. DOI: 10.1093/ajcn/50.5.1231

20 Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M and Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2: Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing *48(1)*: 16-31, 2019. PMID: 30312372. DOI: 10.1093/ageing/afy169

21 De Ceuninck F, Fradin A and Pastoureau P: Bearing arms against osteoarthritis and sarcopenia: when cartilage and skeletal muscle find common interest in talking together. Drug Discov Today *19(3)*: 305-311, 2014. PMID: 23973339. DOI: 10.1016/j.drudis.2013.08.004

22 Santos MJ, Vinagre F, Canas da Silva J, Gil V and Fonseca JE: Body composition phenotypes in systemic lupus erythematosus and rheumatoid arthritis: a comparative study of Caucasian female patients. Clin Exp Rheumatol *29(3)*: 470-476, 2011. PMID: 21640047.

23 Yoshida D, Suzuki T, Shimada H, Park H, Makizako H, Doi T, Anan Y, Tsutsumimoto K, Uemura K, Ito T and Lee S: Using two different algorithms to determine the prevalence of sarcopenia. Geriatr Gerontol Int *14 Suppl 1*: 46-51, 2014. PMID: 24450560. DOI: 10.1111/ggi.12210

24 Miyakoshi N, Hongo M, Mizutani Y and Shimada Y: Prevalence of sarcopenia in Japanese women with osteopenia and osteoporosis. J Bone Miner Metab *31(5)*: 556-561, 2013. PMID: 23515924. DOI: 10.1007/s00774-013-0443-z

25 De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton LJ 3rd, Meunier PJ, Pols HA, Reeve J, Silman A and Tenenhouse A: Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int *16(11)*: 1330-1338, 2005. PMID: 15928804. DOI: 10.1007/s00198-005-1863-y

26 Norman K, Stobäus N, Pirlich M and Bosy-Westphal A: Bioelectrical phase angle and impedance vector analysis—clinical relevance and applicability of impedance parameters. Clin Nutr *31(6)*: 854-861, 2012. PMID: 22698802. DOI: 10.1016/j.clnu.2012.05.008

27 Yamada Y, Buehring B, Krueger D, Anderson RM, Schoeller DA and Binkley N: Electrical properties assessed by bioelectrical impedance spectroscopy as biomarkers of age-related loss of

skeletal muscle quantity and quality. J Gerontol A Biol Sci Med Sci *72(9)*: 1180-1186, 2017. PMID: 28814064. DOI: 10.1093/gerona/glw225

28 Kilic MK, Kizilarslanoglu MC, Arik G, Bolayir B, Kara O, Dogan Varan H, Sumer F, Kuyumcu ME, Halil M and Ulger Z: Association of bioelectrical impedance analysis-derived phase angle and sarcopenia in older adults. Nutr Clin Pract *32(1)*: 103-109, 2017. PMID: 27590205. DOI: 10.1177/0884533616664503

29 Więch P, Sałacińska I, Bazaliński D and Dąbrowski M: Body composition and phase angle as an indicator of nutritional status in children with juvenile idiopathic arthritis. Pediatr Rheumatol Online J *16(1)*: 82, 2018. PMID: 30587206. DOI: 10.1186/s12969-018-0297-y

30 Lukaski HC, Kyle UG and Kondrup J: Assessment of adult malnutrition and prognosis with bioelectrical impedance analysis: phase angle and impedance ratio. Curr Opin Clin Nutr Metab Care *20(5)*: 330-339, 2017. PMID: 28548972. DOI: 10.1097/MCO.0000000000000387

31 Pena NF, Mauricio SF, Rodrigues AMS, Carmo AS, Coury NC, Correia MITD and Generoso SV: Association between standardized phase angle, nutrition status, and clinical outcomes in surgical cancer patients. Nutr Clin Pract *34(3)*: 381-386, 2019. PMID: 29870080. DOI: 10.1002/ncp.10110

32 Gupta D, Lammersfeld CA, Vashi PG, King J, Dahlk SL, Grutsch JF and Lis CG: Bioelectrical impedance phase angle in clinical practice: implications for prognosis in stage IIIB and IV non-small cell lung cancer. BMC Cancer *9*: 37, 2009. PMID: 19175932. DOI: 10.1186/1471-2407-9-37

33 Cereda E, Turri A, Klersy C, Cappello S, Ferrari A, Filippi AR, Brugnatelli S, Caraccia M, Chiellino S, Borioli V, Monaco T, Stella GM, Arcaini L, Benazzo M, Grugnetti G, Pedrazzoli P and Caccialanza R: Whey protein isolate supplementation improves body composition, muscle strength, and treatment tolerance in malnourished advanced cancer patients undergoing chemotherapy. Cancer Med *8(16)*: 6923-6932, 2019. PMID: 31568698. DOI: 10.1002/cam4.2517

34 Pérez Camargo DA, Allende Pérez SR, Rivera Franco MM, Álvarez Licona NE, Urbalejo Ceniceros VI and Figueroa Baldenegro LE: Phase angle of bioelectrical impedance analysis as prognostic factor in palliative care patients at the National Cancer Institute in Mexico. Nutr Cancer *69(4)*: 601-606, 2017. PMID: 28353355. DOI: 10.1080/01635581.2017.1299880

35 Ross R, Léger L, Martin P and Roy R: Sensitivity of bioelectrical impedance to detect changes in human body composition. J Appl Physiol (1985) *67(4)*: 1643-1648, 1989. PMID: 2793764. DOI: 10.1152/jappl.1989.67.4.1643

36 Segal KR, Gutin B, Presta E, Wang J and Van Itallie TB: Estimation of human body composition by electrical impedance methods: a comparative study. J Appl Physiol (1985) *58(5)*: 1565-1571, 1985. PMID: 3997721. DOI: 10.1152/jappl.1985.58.5.1565

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