

# Association between Trunk Muscle Strength, Lumbar Spine Bone Mineral Density, Lumbar Scoliosis Angle, and Skeletal Muscle Volume and Locomotive Syndrome in Elderly Individuals: A Dual-Energy X-ray Absorptiometry Study

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## Abstract:

**Introduction:** The present study aimed to investigate the association between trunk muscle strength, lumbar spine bone mineral density (BMD), lumbar scoliosis angle (LSA), and appendicular skeletal muscle mass index (ASMI) and the severity locomotive syndrome (LS) using dual-energy X-ray absorptiometry (DXA) technology in elderly individuals.

**Methods:** In this cross-sectional study, we enrolled 168 individuals aged >60 years. We measured their trunk muscle strength (flexion and extension) and BMD, LSA, and ASMI using DXA. We defined degenerative lumbar scoliosis (DLS) as LSA  $\geq 10^\circ$  by the Cobb method using the DXA image. The locomotor function was evaluated using the timed up-and-go (TUG) test and the 25-question Geriatric Locomotive Function Scale (GLFS-25) score. Normal locomotor function, LS-1, and LS-2 were defined as a GLFS-25 score of <7,  $\geq 7$  and <16, and  $\geq 16$ , respectively. We compared the three groups, analyzing the associations between all variables and the locomotor function using univariate and multivariate analyses.

**Results:** Although there was no significant difference in sex ratio, BMD, ASMI, and trunk-flexor strength, significant differences were observed in age ( $p < 0.01$ ), the prevalence of DLS ( $p = 0.02$ ), trunk-extensor strength ( $p < 0.01$ ), and trunk-extensor/flexor strength ratio ( $p < 0.01$ ) among the three groups. In multiple regression analyses, the significant risk factors of the TUG test were age ( $\beta = 0.26$ ), body mass index ( $\beta = 0.36$ ), LSA ( $\beta = 0.15$ ), ASMI ( $\beta = -0.30$ ), and trunk-extensor strength ( $\beta = -0.19$ ), whereas the significant factor of the GLFS-25 score was trunk-extensor strength ( $\beta = -0.31$ ).

**Conclusions:** The results indicate that it is clinically important for LS to pay careful attention not only to BMD but also to lumbar scoliosis when DXA examination of the lumbar spine is routinely conducted. Moreover, it is essential to note that trunk-extensor strength is more important than trunk-flexor strength in maintaining locomotor function in elderly individuals.

## Keywords:

Locomotive syndrome, Lumbar scoliosis, Bone marrow density, Trunk muscle strength, Skeletal muscle mass

Spine Surg Relat Res 2020; 4(2): 164-170

dx.doi.org/10.22603/ssrr.2019-0083

## Introduction

Osteoporosis and relevant spinal deformities are often observed in elderly individuals with functional decline. These spinal disorders are associated with locomotor disabilities, including back pain, spinal kyphosis, malalignment of the

sagittal plane and spinal scoliosis, and malalignment of the coronal plane<sup>1-3)</sup>.

In the Japanese society, “locomotive syndrome (LS)” was initially proposed as a new concept called the “musculoskeletal ambulation disability system complex” for elderly individuals<sup>4-6)</sup>. This concept is considered important in Ja-

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Received: September 25, 2019, Accepted: November 5, 2019, Advance Publication: December 3, 2019

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pan's aging society. Several studies have indicated that physical performance<sup>7,8)</sup>, osteoporosis<sup>9)</sup>, sagittal spine imbalance, and sarcopenia<sup>10)</sup> are indicators for LS. "Sarcopenia," defined as a reduction of muscle mass, muscle strength, and physical performance, was associated with functional decline over a 2-year period in elderly Japanese<sup>11)</sup>.

The association between spinal kyphosis and clinical outcomes in the elderly is well documented in several meta-analysis<sup>12)</sup>, and the association between sagittal plane imbalance and LS is also recently reported in Japan<sup>13,14)</sup>. Although Schwab et al. reported a significant association between spinal scoliosis, defined as having a Cobb angle > 10°, and quality of life<sup>15)</sup>, they subsequently found that adult spinal scoliosis was not significantly associated with visual analog scale scores or nutritional status in healthy, elderly volunteers<sup>16)</sup>. Robin et al. previously reported that scoliosis in the elderly rarely became a clinical problem<sup>17)</sup>. Nevertheless, the clinical relevance of radiographical outcome in adult degenerative scoliosis has been well investigated in recent studies<sup>18-20)</sup>. To the best of our knowledge, (1) the association between adult lumbar scoliosis and LS in Japan has not been investigated yet.

In general, bone mineral density (BMD) measurement is used to diagnose osteoporosis and is assessed using dual-energy X-ray absorptiometry (DXA) technology<sup>18,21)</sup>. Over a 10-year period, decreased BMD at the spine and prevalent spinal kyphosis establish the diagnosis of vertebral fractures in Japanese women<sup>19)</sup>. However, (2) the association between BMD at the lumbar spine and LS is still unknown. Currently, DXA can evaluate not only BMD but also whole body composition, such as muscles and fats<sup>20)</sup> and lumbar scoliosis angle (LSA)<sup>22)</sup>, enabling the simultaneous evaluation of LSA, BMD, and skeletal muscle volume (SMV) and their clinical relevance.

Although the 25-question Geriatric Locomotive Function Scale (GLFS-25) was established to assess the presence or the severity of LS<sup>23)</sup>, this comprehensive tool is often influenced by psychosocial disabilities based on patient-reported outcome measure. Thus, a physical performance test is ideal concomitant with the GLFS-25 to assess locomotor function. The National Institute of Clinical Evidence guidelines recommend the timed up-and-go (TUG) test for the assessments of gait and balance in elderly people<sup>24)</sup>, and some studies revealed a close link between LS and TUG<sup>8,14,25)</sup>. Imagama et al. reported that gait speed in elderly people was associated not only with spinal deformities but also with back muscle strength. Therefore, trunk muscle strength assessment was needed to evaluate the associations between LS, locomotor function, and physiological parameters measured by DXA examination.

To address both questions of (1) and (2), this study aimed to investigate the association between trunk muscle strength, lumbar scoliosis, BMD at lumbar spine, and SMV and locomotor function in elderly individuals using DXA technology.

## Materials and Methods

### Subject enrollment

This was a cross-sectional study conducted in a single institute. In our center, users are well and are able to exercise alone regardless of several concurrent comorbidities, such as hypertension, hyperlipidemia, and various musculoskeletal disorders, with or without medication. They usually perform supervised physical exercises, depending on their condition, to promote their health. They undergo an annual medical checkup to assess their health. We used these opportunities to conduct a research study that investigated the association between LS and degeneration or osteoporosis of the lumbar spine in elderly individuals from September 2014 to December 2016. We recruited participants who were interested in this study. Participants aged ≥60 years and those who could perform physical exercises to treat their diseases or to promote their health were included. Participants who underwent lumbar operation with metal implant and those who were not able to continue performing exercises alone because of cognitive disorders, suspected dementia, or severe pulmonary and heart abnormalities diagnosed during annual checkup were excluded.

A total of 169 elderly individuals voluntarily participated in this study. This study was ethically approved by the Research Ethics Committee of our institute. After being informed of the purpose and protocols of the study, the participants provided written informed consent before undergoing any examinations.

### Epidemiological and morphological characteristics

First, we checked the epidemiological background of the participants in terms of age and sex to assess their contribution to each parameter. Second, height, weight, and body mass index (BMI) of the participants were assessed as morphological parameters.

### Dual-energy X-ray absorptiometry scans

We measured both the LSA and BMD of the lumbar spine using a DXA scanner (QRD 4500<sup>®</sup>, Hologic, Inc.)<sup>26)</sup>. The DXA scans of the lumbar spine were routinely performed with patients assuming a supine position. The LSA in the coronal plane was measured using the Cobb method between L2 and L4 if a curve was evident. Two examiners (H.T and J.S) independently measured the scoliosis angles twice. Intra- and interobserver reliability was calculated for the intra- and interclass correlation coefficient (ICC)<sup>27)</sup>. We confirmed that the intra-observer reliability of LSA was very good (ICC (1,1) = 0.96, 0.98), and the interobserver reliability was also very high (ICC (2,2) = 0.98). Therefore, we adapted the mean of LSAs measured by Observer 1 (H.T). Subsequently, we classified the participants into dichotomous subgroup by LSA according to the Schwab criteria<sup>15,16)</sup>, positive for degenerative lumbar scoliosis (DLS) (LSA was >10°) or negative for DLS (LSA was <10°).

Simultaneously, BMD in the lumbar spine (L2, L3, and L4) was assessed using the conventional anteroposterior view method. Osteoporosis was defined using the criteria of the World Health Organization (T-score < -2.5)<sup>28</sup>. Furthermore, we measured the appendicular skeletal muscle mass (ASM) of each participant to determine SMV. ASM was calculated by summing the muscle masses of the four limbs, assuming that all nonfat and non-bone mass was skeletal muscle. For normalization, the appendicular skeletal muscle index (ASMI) was defined as ASM/height<sup>2</sup> (kg/m<sup>2</sup>)<sup>29</sup>.

### Trunk muscle strength

Isometric muscle strength was measured using a muscle function analyzing device (Cybex Norm<sup>®</sup>, Cybex Co., Ltd.). A maximum 5-s isometric strength (Nm) was measured once for flexion and extension in both trunk muscles with patients assuming a standing position, and an average of those values was adapted. The trunk-extensor/flexor ratio was used to indicate trunk muscle balance<sup>30</sup>.

### Physical performance test

The TUG test was used to measure functional capacity in the subjects. We measured the time it took a participant to rise from a standard chair (46-cm seat height from the ground), walk a distance of 3 m, turn around, walk back to the chair, and sit down<sup>31</sup>. All participants performed two trials, and the superior time was adapted.

### Severity of locomotive syndrome (LS)

Recently, a quantitative screening tool called the 25-question Geriatric Locomotive Function Scale (GLFS-25) has been developed to measure the severity of LS<sup>23</sup>. The GLFS-25 is a self-administered, relatively comprehensive measure that consists of 25 items, including 4 questions regarding pain during the last month, 16 questions regarding activities of daily living during the last month, 3 questions regarding social functions, and 2 questions regarding mental health status during the last month. These 25 items are graded on 5 points from no impairment (0 points) to severe impairment (4 points) and, subsequently, arithmetically added to produce a total score (minimum 0 and maximum 100). A higher score is considered to be associated with a higher severity of LS. Using the recently determined GLFS-25 cutoff value, participants were divided into three groups. A normal locomotor function was defined by a GLFS-25 score of <7, LS stage 1 (LS-1) was defined by a GLFS-25 score of ≥7 and <16, and LS stage 2 (LS-2) was defined by a GLFS-25 score of ≥16<sup>32</sup>.

### Statistical analyses

We compared the three different LS severity groups using the Kruskal-Wallis test followed by the *post hoc* Steel-Dwass method for the continuous variables or  $\chi^2$  test for the categorical variables. We analyzed the association between the GLFS-25 score and each variable using Spearman's correlation coefficient for univariate analysis. Further analysis

using a stepwise multiple linear regression model was conducted to predict the contributors for the TUG or the GLFS-25 score. Independent variables (sex, age, BMI, BMD, T-score, osteoporosis(+), ASMI, LSA, DLS(+), trunk-flexor strength, trunk-extensor strength, and extensor/flexor strength ratio) were included in the multiple regression model if a potential association with the TUG or the GLFS-25 response was at  $p < 0.1$  in the univariate analysis. Statistical analyses were performed using the Statistical Package for the Social Sciences software (version 25.0J; IBM Corp., Armonk, NY). A significance level of  $p < 0.05$  was considered statistically significant.

## Results

### Comparison of each parameter according to the LS severity

In 168 participants, 34% and 22% had LS-1 and LS-2, respectively. Table 1 presents the mean ± standard deviation of each variable in the groups divided by the GLFS-25 cutoff value. There were significant differences in age, but not in BMI, BMD, ASMI, or sex, between the groups. Although there was no significant difference in trunk-flexor strength, significant differences were observed in trunk-extensor strength and trunk-extensor/flexor strength ratio between the groups. Additionally, there were significant differences in the prevalence of DLS at 10.8% in the normal group, 17.5% in the LS-1 group, and 32.4% in the LS-2 group.

### Correlation analysis between each of the variables

The correlation coefficients ( $\rho$ ) between each measured variable are presented in Table 2. The TUG outcomes and the GLFS-25 scores revealed a significant positive association with female, aging, LSA, and DLS (+) and significant inverse association with trunk-extensor strength and flexor/extensor strength ratio. Furthermore, the TUG revealed an inverse association with trunk-flexor strength.

Multiple linear regression analysis revealed that the significant risk factors of TUG were aging, BMI, LSA, ASMI, and trunk-extensor strength with the coefficient of determination ( $R^2$ ) = 0.25. Moreover, the significant risk factor of GLFS-25 score in multiple linear regression analysis was trunk-extensor strength with  $R^2$  = 0.14. (Table 3)

## Discussion

In this study, we investigated the associations between LSA, lumbar BMD, ASMI, and trunk muscle strengths and locomotor function in elderly Japanese individuals using DXA technology. Our results revealed that the degree of LSA was more likely associated with the severity of locomotor function than that of BMD, implying that it is clinically important to pay careful attention to lumbar scoliosis, regardless of intact BMD, for elderly individuals when DXA examination of the lumbar spine is routinely performed.

**Table 1.** Comparison of Variables between Normal, LS1, and LS2.

Variables	Normal	Locomotive syndrome (LS)		p-value
		Stage 1 (LS1)	Stage 2 (LS2)	
Number of subjects, N (%)	74	57	37	
Demographic parameters				
Sex, male/female	22/52	15/42	5/32	
Age (yr),	69.26 (5.12)	71.56 (6.26)	73.59 (7.26) **	<0.01
BMI (kg/m <sup>2</sup> ),	22.84 (2.75)	23.68 (4.28)	23.30 (3.06)	0.78
DXA parameters				
BMD (L2- L4) (g/cm <sup>2</sup> ),	0.92 (0.19)	0.92 (0.18)	0.93 (0.20)	1.00
T-score,	-0.88 (1.62)	-0.91 (1.50)	-0.79 (1.77)	1.00
Osteoporosis, N (%)	8 (10.8)	9 (15.8)	5 (13.5)	0.70
ASMI (kg/m <sup>2</sup> ),	6.54 (0.83)	6.59 (0.93)	6.28 (0.88)	0.19
LSA (°),	2.65 (4.39)	3.96 (6.72)	6.38 (8.94)	0.14
Lumbar scoliosis, N (%)				
Normal (LSA<10), N (%)	66 (89.2)	47 (82.5)	25 (67.6)	0.02
Scoliosis (LSA≥10), N (%)	8 (10.8)	10 (17.5)	12 (32.4)	
Physical function				
TUG (sec)	5.09 (0.64)	5.76 (0.96) **	6.73 (1.59) **, \$\$	<0.01
Trunk-flexor strength (Nm),	95.65 (42.23)	93.00 (39.55)	80.54 (38.14)	0.15
Trunk extensor strength (Nm)	143.15 (69.41)	130.05 (55.47)	93.35 (52.65) **, \$\$	<0.01
Trunk extensor/flexor ratio	1.56 (0.50)	1.49 (0.51)	1.23 (0.53) **	<0.01

Abbreviations: BMI, body mass index; BMD, bone mineral density; ASMI, appendicular skeletal mass index; LSA, lumbar scoliosis angle; TUG, timed-up-and-go test; GLFS-25, Geriatric Locomotive Function Scale. Each variable is compared across the three groups using  $\chi^2$  test for categorical variables, or using the Kruskal-Wallis test with the *post-hoc* Steel-Dwass method for continuous variables (\*p<.05, \*\*p<.01 for normal vs LS2, \$p<.05, \$\$p<.01 for LS1 vs LS2).

**Table 2.** Correlation Coefficients between Each of the Variables.

	Sex	Age	BMI	BMD	T-score	Porosis (+)	ASMI	LSA	DLS (+)	TUG	Flexor-s	Extensor-s	E/F ratio	GLFS25
Sex	1.00	-0.07	-0.19*	-0.50**	-0.45**	0.14	-0.59**	0.14	0.16*	0.17*	-0.43**	-0.31**	0.15	0.17*
Age		1.00	0.00	0.02	0.01	0.05	-0.02	0.30**	0.21**	0.35**	-0.08	-0.25**	-0.23**	0.23**
BMI			1.00	0.30**	0.31**	-0.26**	0.64**	-0.17*	-0.17*	0.11	0.11	0.00	-0.22*	0.04
BMD				1.00	0.99**	-0.58**	0.49**	0.10	0.09	-0.09	0.38**	0.21**	-0.19*	0.02
T-score					1.00	-0.58**	0.47**	0.10	0.10	-0.09	0.37**	0.20*	-0.19*	0.02
Porosis (+)						1.00	-0.23**	-0.05	0.00	0.03	-0.22**	-0.15	0.09	0.01
ASMI							1.00	-0.09	-0.12	-0.11	0.35**	0.17	-0.26**	-0.13
LSA								1.00	0.76**	0.21**	0.02	-0.12	-0.19*	0.17*
DLS (+)									1.00	0.21**	0.06	-0.13	-0.19*	0.22**
TUG										1.00	-0.23**	-0.38**	-0.25**	0.50**
Flexor-s											1.00	0.71**	-0.28**	-0.13
Extensor-s												1.00	0.40**	-0.29**
E/F ratio													1.00	-0.23**
GLFS25														1.00

Value: Spearman's correlation coefficient \* p<.05, \*\* p<.01

Abbreviations: BMI, body mass index; BMD, bone mineral density; ASMI, appendicular skeletal mass index; LSA, lumbar scoliosis angle; DLS, degenerative lumbar scoliosis; TUG, timed-up-and-go test; Flexor-s, trunk-flexor strength; Extensor-s, trunk-extensor strength; E/F ratio, trunk-extensor/flexor strength ratio; GLFS-25, Geriatric Locomotive Function Scale

Moreover, our results indicate that it is important to note that trunk-extensor strength is more important than trunk-flexor strength in maintaining locomotor function in elderly individuals.

Loss of BMD and degenerative vertebral deformity in the lumbar spine are usually separately examined in the elderly

on medical care because they are independent biomarkers that predict functional decline in the future. According to the previous studies, decreased physical performance is associated with a low BMD<sup>33,34</sup>. Although several authors have reported that a low BMD was associated with LS using calcaneal quantitative ultrasound (QUS)<sup>9,35,36</sup>, to the best of our

**Table 3.** Multiple Linear Regression Analysis for Locomotive Function.

Outcome	Independent variables	R <sup>2</sup>	B	95% CI for B	$\beta$	p-value
TUG	Age	0.25	0.05	0.02, 0.08	0.26	<b>&lt;0.001</b>
	BMI		0.13	0.06, 0.19	0.36	<b>&lt;0.001</b>
	ASMI		-0.41	-0.17, 11.37	-0.30	<b>&lt;0.001</b>
	LSA		0.03	0.05, 4.25	0.15	<b>0.041</b>
	Trunk extensor		0.00	-0.01, 0.00	-0.19	<b>&lt;0.01</b>
GLSF-25	Age	0.14	0.18	-0.62, 6.59	0.14	0.10
	Sex		2.99	-0.05, 0.41	0.12	0.13
	LSA		0.15	-0.08, 0.37	0.10	0.20
	Trunk flexor		0.04	-0.01, 0.09	0.19	0.10
	Trunk extensor		-0.04	-0.02, -0.08	-0.31	<b>&lt;0.001</b>

R<sup>2</sup>: Coefficient of determination, B: Unstandardized coefficients,  $\beta$ : Standardized coefficients  
 Abbreviations: BMI, body mass index; ASMI, appendicular skeletal mass index; LSA, lumbar scoliosis angle; TUG, timed-up-and-go test; GLFS-25, Geriatric Locomotive Function Scale

knowledge, no previous study has investigated the association between BMD of the lumbar spine using the DXA and LS. The current study initially demonstrated that lumbar spine BMD was not associated with LS, which was one of the strengths in this study. The possible reasons to these inconsistencies are discussed as follows. First, sampling bias would affect the results of each study because approximately half of our samples were considered as out of the LS range. Second, a measurement variation between calcaneal QUS and DXA has been discussed in the previous literatures, and a systematic review concluded that QUS of the calcaneus still cannot be used to establish the diagnosis of osteoporosis using DXA<sup>37</sup>. Third, it is well known that the presence of spondylophytes and degenerative intervertebral discs, which are commonly observed in the DLS, overestimates DXA BMD measurements<sup>38,39</sup>. Therefore, we assume that the BMD of the participants with the DLS in our study seems to show an evident increase due to sclerotic change<sup>22</sup>. Hence, studies involving a whole body scan to assess BMD using DXA will be required for LS in the future.

Regarding the physiological and physical parameters, we found that scoliosis parameter was associated with LS, and LSA was a significant risk factor of the TUG, but not the GLSF-25. As the TUG test is more considered to predict pure locomotor capacity than the GLSF-25 score, the severity of lumbar scoliosis has to be considered as a significant risk factor to locomotor decline. Moreover, we found that trunk muscle strength was a potential predictor for not only locomotor capacity but also the BMD in the lumbar spine. Previous studies have reported that a decline in back muscle power was associated with LS<sup>40</sup> and 10-m gait speed<sup>7</sup>, which is consistent with the study results. Trunk muscle balance with predominance of extensor over flexor muscles is associated with better physical capacity<sup>41</sup> and less back pain<sup>31</sup>. Masaki et al. reported that maximal walking speed was negatively associated with the thickness of lumbar erector

spinae muscles in middle-aged and elderly women<sup>42</sup>. Some authors have recently reported that the ratios of trunk flexion to extension in healthy untrained adults were usually lower than those in athletes<sup>43</sup>, suggesting that a superiority of trunk-extensor to flexor strength is associated with high performance even in the healthy participants. Moreover, our results revealed that trunk-extensor strength was associated with not only physical performance but also LS severity after controlling several confounders, and age was associated with trunk-extensor/flexor strength ratio. Thus, an instruction of back muscle training is important for elderly individuals to prevent from developing LS.

Meanwhile, we found that trunk-flexor strength is possibly associated with lumbar BMD; however, a further study is required to confirm this association by controlling the possible confounders, such as age, sex, underlying disease, and mediations between them.

Interestingly, our results revealed that there was no significant association between ASMI and LS severity. Although some authors had found an association between sarcopenia and LS, they defined sarcopenia according to the consensus report<sup>10,11</sup>, we assessed SMV only by ASMI without grip strength and walking speed. Although our results indicate a weak association between ASMI and TUG in multivariate analysis, a discordant association between ASMI and LS in our study might be explained by the non-linear association between muscle strength and SMV<sup>44,45</sup> or sampling bias. Clinical significance of muscle mass decrease for LS should be discussed in further study.

This study has some limitations. First, we measured the LSA using only the DXA method. The LSA has traditionally been measured using an X-ray method with participants assuming a standing position<sup>15</sup>. On the contrary, in this study, LSA was measured using the DXA method with participants assuming a supine position. The LSA using the DXA method was reported to be approximately 5° less than

that using the X-ray method<sup>22)</sup>. Moreover, we measured only the LSA within the L2-L4; hence, thoracolumbar malalignment in both the coronal and sagittal planes could not be assessed in this study. Therefore, our result might have been different if the scoliosis angle had been measured using the X-ray method and had been assessed until the thoracolumbar curve, including sagittal imbalance. Second, basic information regarding musculoskeletal disorders of the participants, such as osteoarthritis, which was related to LS<sup>4,6)</sup>, was not assessed in this study. Therefore, other musculoskeletal disorders, concurrent comorbidities such as metabolic syndrome, and medication uses must relatively affect the results of this study. Moreover, the severity of LS defined using the GLFS-25 questionnaire was associated not only with physical capacity but also with psychosocial status<sup>8,46)</sup>. Therefore, multifactorial factors surrounding the participants might also affect the results. Third, this was only a cross-sectional study. Hence, a longitudinal study is required to confirm the association between LSA and LS in the future.

In conclusion, the association between LSA, lumbar BMD, ASMI, and trunk muscle strengths and LS was investigated in elderly Japanese individuals using the DXA technology. The results indicate that it is clinically important for LS to pay careful attention not only to BMD but also to lumbar scoliosis when DXA examination of the lumbar spine is routinely performed. Moreover, it is important to note that trunk-extensor strength is more important than trunk-flexor strength in maintaining locomotor function in elderly individuals.

**Conflicts of Interest:** The authors declare that there are no relevant conflicts of interest.

**Author Contributions:** Takenaka H. conceived and designed the study, analyzed the data, and prepared the draft and tables.

Ikemoto T. conceived and designed the study, collected data, analyzed the data, and prepared the draft and tables.

Suzuki J. analyzed the data and prepared the draft and tables.

Inoue M. & Arai Y.C. collected the data and prepared the draft and tables.

Ushida T. & Deie M. designed the study and reviewed drafts of the paper.

Kamiya M. conceived and designed the study, prepared the draft and tables, and reviewed drafts of the paper.

**Informed Consent:** Informed consent was obtained by all participants in this study.

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