Relationship between serum creatinine to cystatin C ratio and subclinical atherosclerosis in patients with type 2 diabetes

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ABSTRACT
Introduction Sarcopenia index (SI), calculated by (serum creatinine/serum cystatin C)×100, is reported to be associated with sarcopenia. Few studies reported the association between SI and subclinical atherosclerosis. We evaluated the association between SI and subclinical atherosclerosis, assessed by brachial-ankle pulse wave velocity (baPWV).

Research design and methods One hundred seventy-four patients with type 2 diabetes were included in this cross-sectional study. The relationship between SI and baPWV was assessed by Pearson’s correlation coefficient. To calculate area under the receiver operator characteristic (ROC) curve (AUC) of SI for the presence of subclinical atherosclerosis, which was defined as baPWV >1800 cm/s, ROC analysis was performed. Logistic regression analyses were performed to assess the effect of SI on the prevalence of subclinical atherosclerosis adjusting for covariates.

Results Mean age, duration of diabetes, baPWV, and SI were 66.9 (10.1) years, 17.7 (11.6) years, 1802 (372) cm/s, and 77.6 (15.8), respectively. There was an association between SI and baPWV (men; r=−0.25, p=0.001, and women; r=−0.37, p=0.015). The optimal cut-off point of SI for the presence of subclinical atherosclerosis was 77.4 (sensitivity=0.72, specificity=0.58, p<0.001, AUC 0.66 (95% CI: 0.57 to 0.74)). In addition, SI was associated with the prevalence of subclinical atherosclerosis (adjusted OR 0.95, 95% CI: 0.91 to 0.99, p=0.015).

Conclusions SI is associated with the prevalence of subclinical atherosclerosis in patients with type 2 diabetes.

INTRODUCTION
Among patients with diabetes, cardiovascular disease (CVD) is an important cause of morbidity and mortality;1,2 it is reported that one of the major risk factor for CVD is atherosclerosis, which is a risk for peripheral arterial disease (PAD) and non-embolic ischemic stroke.3 Type 2 diabetes (T2D) is known to cause atherosclerosis.4 Subclinical atherosclerosis has been reported to be a risk for cardiovascular events in patients with diabetes.5,6

Therefore, screening for subclinical atherosclerosis is useful for identifying high-risk patients including patients with diabetes.7 Sarcopenia is becoming an important consideration in older patients with T2D, because the number of older patients with diabetes is increasing.8 Sarcopenia is reported to be associated with risk of cardiovascular disease and mortality.9-12 Serum creatinine/serum cystatin C ratio, called as sarcopenia index (SI), is used as a surrogate marker for sarcopenia.13-16 Previous studies show that there is an association SI with incidence CVD events and mortality.17,18

Pulse wave velocity (PWV) is developed as a marker of arterial stiffness19 and is expressed as the severity of vascular damage;20 it is a useful tool to detect subclinical atherosclerosis.21 In particular, brachial-ankle PWV (baPWV) is useful to detect subclinical
Cardiovascular and metabolic risk

However, few studies revealed the association between SI and subclinical atherosclerosis. Therefore, in this cross-sectional study, we used baPWV to evaluate the relationship between SI and subclinical atherosclerosis.

MATERIALS AND METHODS

Study participants

In this cross-sectional study, we extracted the data from the KAMOGAWA-DM cohort study, which is an ongoing prospective cohort study. To clarify the natural history of patients with diabetes, we invited all patients with diabetes who were outpatients of Department of Endocrinology and Diabetes of Kyoto Prefectural University of Medicine Hospital (Kyoto, Japan) or Department of Diabetes of Kameoka Municipal Hospital (Kameoka, Japan) to participate in the KAMOGAWA-DM cohort study and enrolled those who agreed to participate. In this study, we extracted the data of patients who were registered from November 2016 to December 2017. Exclusion criteria were as follows: non-T2D, no data of baPWV, no data of CysC, renal dysfunction (defined as serum Cre >2.0 mg/dL), missing data of body weight, history of CVD, including heart failure, unstable angina, myocardial infarction, stroke, and peripheral artery disease (defined as ankle-brachial index <0.9).

Data collection and measurements

Body mass index (BMI) was assessed as weight (kg) divided by height² (m²). Using a standardized self-administered questionnaire, the data of habitual alcohol consumption (consuming alcohol daily or not), smoking status (current smoker or not), and exercise habit (exercise of some kind at least once a week or not) were obtained. Usage of medications for hypertension and diabetes, including insulin, sulfonylurea, glinide, dipeptidyl peptidase-4 inhibitors, biguanide, thiazolidine, alpha-glucosidase inhibitors, sodium glucose co-transporter 2 inhibitors (SGLT2i), glucagon-like peptide-1 receptor agonist (GLP-1RA), and dyslipidemia, including statin, were obtained from medical records.

Serum was collected after an overnight fast. Serum Cre and CysC levels were measured. SI was calculated as (serum Cre divided by serum CysC)×100.

After at least 5 min of rest in the supine position, the baPWV was measured using a Colin Waveform analyser (PWV/ABI, Colin Medical Technology, Komaki, Japan) as well as both arm and ankle blood pressure. The details of this method have been described elsewhere. The Pearson’s correlation coefficients of interobserver and intraobserver reproducibility were r=0.98 and r=0.87, respectively. The higher baPWV, with both sides measured, was used as representative for each patient. At same time, the lower ABI, with both sides measured, was used as represent...
Cardiovascular and metabolic risk

used as representative for each patient. A $baPWV > 1800$ cm/s was defined as subclinical atherosclerosis.  

**Statistical analysis**

The statistical analyses were carried out using the JMP V.13.2.1 software (SAS Institute, Cary, North Carolina, USA). Statistical significance was set at $p$ value $< 0.05$.

Continuous variables were expressed as mean (SD) and categorical variables were expressed as percentage (%) (number). The relationships between CysC, Cre, or SI and $baPWV$ were evaluated by Pearson’s correlation coefficient. The receiver operating characteristic (ROC) analysis was used to calculate area under the ROC curve (AUC) of CysC, Cre, or SI for the presence of subclinical atherosclerosis. Then, logistic regression analyses were performed to evaluate the effect of CysC, Cre, or SI on the presence of subclinical atherosclerosis, adjusting for age, sex, BMI, smoking status, exercise habit, systolic blood pressure, hemoglobin $A_1c$ (HbA1c), antihypertensive medication, insulin, SGLT2i, GLP-1RA, and statin usage. Furthermore, subanalyses were performed for patients aged $\geq 65$ years.

**RESULTS**

Five hundred sixty-three patients were included in this cross-sectional study. After excluding 389 patients, 174 patients were selected for this study (figure 1). The clinical characteristics of this study patients are shown in table 1. Mean age, duration of diabetes, and $baPWV$ were 66.9 (10.1) years, 17.7 (11.6) years, and 1802 (372) cm/s, respectively. The proportion of subclinical atherosclerosis was $43.7\%$ ($n=76$).

Figure 2 Correlation between cysteine C (CysC), creatinine (Cre), or sarcopenia index and brachial-ankle pulse wave velocity (PWV). (A–C) Correlation between cysteine C, creatinine, or sarcopenia index and brachial-ankle PWV among men. (D–F) Correlation between cysteine C, creatinine, or sarcopenia index and brachial-ankle PWV among women.

Data were expressed as mean (SD) or number (%). SI was calculated as $(\text{serum Cre divided by serum cystatin C}) \times 100$.

Figure 3 Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) of cysteine C, creatinine, or sarcopenia index for subclinical atherosclerosis. (A) All patients. Red line represents cysteine C (AUC $0.62$, 95% CI $0.53$ to $0.70$), green line represents creatinine (AUC $0.49$, 95% CI $0.40$ to $0.57$), and blue line represents sarcopenia index (AUC $0.66$, 95% CI $0.57$ to $0.74$). The $p$ values of difference of AUC of cysteine C and creatinine, cysteine C and sarcopenia index, and creatinine and sarcopenia index were $<0.001$, $0.449$, and $0.023$, respectively. (B) Patients aged $\geq 65$ years. Red line represents cysteine C (AUC $0.53$, 95% CI $0.43$ to $0.64$, $p=0.071$), green line represents creatinine (AUC $0.53$, 95% CI $0.43$ to $0.64$), and blue line represents sarcopenia index (AUC $0.61$, 95% CI $0.51$ to $0.71$). The $p$ values of difference of AUC of cysteine C and creatinine, cysteine C and sarcopenia index, and creatinine and sarcopenia index were $0.992$, $0.272$, and $0.071$, respectively.
Cardiovascular and metabolic risk covariates, SI was related to the prevalence of subclinical atherosclerosis (OR 0.95, 95% CI: 0.91 to 0.99, p=0.015), whereas CysC (OR 1.51, 95% CI: 0.24 to 9.60, p=0.658) or Cre (OR 0.26, 95% CI: 0.02 to 2.80, p=0.263) was not.

Subanalyses of patients aged ≥65 years were also performed. SI was associated with baPWV, whereas CysC or Cre was not associated with baPWV in both sexes (figure 4). In addition, the AUC of SI for the prevalence of subclinical atherosclerosis (0.61 (95% CI: 0.51 to 0.71)) tended to be superior to that of Cre (0.53 (95% CI: 0.43 to 0.64), p=0.071). Furthermore, the optimal cut-off point of SI for the prevalence of subclinical atherosclerosis was 77.2 (sensitivity=0.70, specificity=0.54, p=0.044) (figure 3B). SI was related to the prevalence of subclinical atherosclerosis (OR 0.94, 95% CI: 0.89 to 0.99, p=0.017), whereas CysC (OR 2.45, 95% CI: 0.27 to 21.9, p=0.424) or Cre (OR 0.43, 95% CI: 0.03 to 5.96, p=0.531) was not, after adjusting for covariates (table 2).
DISCUSSION

In this study, we investigated the association between SI and subclinical atherosclerosis in patients with type 2 diabetes mellitus (T2DM) and clarified that there is an association of SI with baPWV and the prevalence of subclinical atherosclerosis.

A recent meta-analysis showed that the proportion of sarcopenia in patients with T2DM was higher than that in people without T2DM. Sarcopenia is reported to be associated with a risk of atherosclerosis, and mortality. Recent studies showed that SI is useful for sarcopenia and physical activity levels. In addition, SI is reported to be associated with incident CVD and mortality. However, little is known about the association between SI and subclinical atherosclerosis. Recently, Shin revealed that there was an association between SI and carotid plaque score in patients with T2DM.

A possible explanation for the association between SI and subclinical atherosclerosis is discussed below. In sarcopenia, the balance between muscle catabolism and synthesis is disrupted due to suppression of the proliferative signaling pathway of skeletal muscle cells and overactivation of the apoptotic signaling pathway. Malnutrition, physical inactivity, oxidative stress, hormonal changes, inflammation, insulin resistance, autophagy, and apoptosis have been implicated in the development of both CVD and sarcopenia. Sarcopenia and CVD are closely related through insulin resistance, inflammation, and oxidative stress. For example, increased interleukin (IL)-6, which is associated with the degree of atherosclerosis in older patients, encourages the catabolism of skeletal muscle and muscle atrophy. Increased reactive oxygen species (ROS) causes vasoconstriction and encourages arterial hypertension, promotes the formation of atherosclerotic plaques, and causes vascular endothelial dysfunction. The accumulation of ROS leads to sarcopenia by promoting muscle hydrolysis; at the same time, it affects muscle protein synthesis via nitrification. In addition, high palmitic acid intake leads to muscle atrophy and CVD through increasing ROS. These mechanisms are involved in SI and subclinical atherosclerosis.

The limitations of our study should be mentioned. First, since our study is a cross-sectional study, casual nexus is unknown and further follow-up study is needed to clarify this relationship. Second, relatively small number of participants, so there is a possibility that it may not accurately represent the study population. The characteristics, including sex, smoking, BMI, and HbA1c, were not different between analysis group and exclusion group (men, 56.3% vs 60.9%, p=0.304; smoker, 14.4% vs 13.3%, p=0.741; BMI, 23.5 (3.5) vs 24.1 (4.3) kg/m², p=0.304; and HbA1c, 7.3 (0.9) vs 7.2 (0.9) %, p=0.163), although age of the analysis group was lower than that of exclusion group (66.9 (10.1) vs 70.4 (10.2) years, p<0.001). Third, measurement of markers of inflammation and oxidative stress, such as serum IL-6, 4-hydroxynonenal, and urinary 8-OH-dG may support our hypothesis. Unfortunately, however, we did not measure these markers. Lastly, we only included Japanese patients, thus, it is uncertain that the results of this study generalized to non-Japanese populations, especially non-Asian populations.

In conclusion, SI is associated with the presence of subclinical atherosclerosis in patients with T2DM and this has implications for the management of these patients. Furthermore, large-scale prospective studies are needed to clarify the causal nexus.

Contributors YH designed the study, researched, analyzed, and interpreted data and wrote manuscript; FT researched and interpreted data and contributed to discussion; TOk, T0s, TS, HO, SM, EU, NN, and MA researched data and contributed.

Figure 4 Correlation between cysteine C (CysC), creatinine (Cre), or sarcopenia index and brachial-ankle pulse wave velocity (PWV) among patients aged ≥65 years. (A–C) Correlation between cysteine C, creatinine, or sarcopenia index and brachial-ankle PWV among men. (D–F) Correlation between cysteine C, creatinine, or sarcopenia index and brachial-ankle PWV among women.
Cardiovascular and metabolic risk

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Patient consent for publication
Not applicable.

Ethics approval
The Ethical Committee of Kyoto Prefectural University of Medicine approved this study (No. RBMR-E-466-5) and written informed consent was obtained from all participants.

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Data are available on reasonable request.

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