Original article

Serum cystatin C level is associated with locomotive syndrome risk and can be an early predictor in community-living people: The Yakumo study

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Abstract

Objectives: The locomotive syndrome (LS) risk has been recently proposed as a criterion for evaluating physical ability. Serum cystatin C level is an early renal function marker and a cardiovascular disease predictor. This study aimed to evaluate the relationship between serum cystatin C level and LS risk.

Methods: We enrolled 54 participants and conducted the two-step test, stand-up test, 25-question geriatric locomotive function scale, LS risk test, Timed Up and Go test, back muscle strength, grip strength, blood test, and serum cystatin C level measurement. A comparative study was conducted in participants with and without LS risk and in subgroups aged <60 and ≥60 years.

Results: No significant difference was found in the serum cystatin C level in subgroups aged <60 years and without LS risk. However, it was significantly higher in subjects with LS risk and aged ≥60 years. The area under the curve of the serum cystatin C level for LS risk was 0.824.

Conclusions: The serum cystatin C level is significantly related to LS risk and can be an early predictor. In middle-aged and elderly people with high serum cystatin C levels, it is strongly recommended to enforce LS risk test and intervention.

Introduction

With the rise of the aging population, musculoskeletal problems are becoming increasingly prevalent. In addition, severe cases and multiple disease complications are often difficult to manage with the existing guidelines. A new approach is necessary to promptly detect and address these problems in more people.

In 2007, the Japanese Orthopedic Association (JOA) proposed the concept of "locomotive syndrome" (LS), which is a condition where people from high-risk groups with musculoskeletal diseases require high demand for nursing care at some point [1,2]. In 2013, the JOA proposed the following three tests to evaluate the risk of LS: the two-step test, stand-up test, and 25-question geriatric locomotive function scale (GLFS-25) [3]. From the test results, the mobility and stage of LS can be determined. The LS risk is categorized into two stages (1 and 2) [3–5]. Of note, LS risk stage 1 indicates that the movement function has begun to decline and that measures to prevent deterioration to LS should be implemented. Therefore, early diagnosis of LS risk is very important for the treatment of these conditions.

Recent studies have shown that the serum cystatin C level is not only a sensitive indicator of early renal function but also a strong independent predictor of cardiovascular disease, diabetes mortality, and all-cause mortality [6,7]. However, the association between serum cystatin C level and LS risk has not been clarified. Thus, the purpose of this study was to investigate the relationship between serum cystatin C level and LS risk in community-living people and whether serum cystatin C level may be useful as an early predictor of LS risk.

Materials and methods

Participants

The subjects were healthy Japanese volunteers who underwent a basic health checkup supported by the local governments in 2016. Since 1982, this checkup that comprised of voluntary orthopedic and physical function examinations, internal medical examinations, and psychological tests has been conducted every August in the town of Yakumo in a rural area of southern Hokkaido, Japan [8–11]. Individuals with the following test data were included: (1) serum cystatin C level measured with a blood test; (2) two-step and stand-up tests for physical function and grip strength, back muscle strength, and 3-m Timed Up and Go test (TUG) for physical function; and (3) answers to all questions in the GLFS-25 [12]. The exclusion criteria were as follows: a history of spinal, hip, and knee surgery; severe knee injury; severe osteoarthritis; history of fracture of the hip and spine; and treatment of diabetes, kidney disease, and heart disease.

Among the 555 participants who underwent this checkup in 2016, 294 participants were selected to undergo the LS risk test and physical performance test. Of these 294 participants, measurement of the serum cystatin C level was carried out in 54 participants who gave their written informed consent for sample collection. Therefore, 54 participants were ultimately included in the study. The study protocol was approved by the University Committee on Ethics in Human Research and by the Institutional Review Board of Nagoya University Graduate School of Medicine. All participants provided written informed consent and the study protocol was approved by the Institutional Review Board of Nagoya University Graduate School of Medicine. The study procedures were carried out in accordance with the principles of the Declaration of Helsinki.

Blood test and measurement of serum cystatin C level

We obtained venous blood samples and performed a blood test. In this study, creatinine, which is one of the kidney function markers, and C-reactive protein (CRP), which is a marker of inflammation, were used for

analysis. The serum cystatin C level was measured using the latex agglutination turbidimetric immunoassay (LA) method (LSI Medience Corporation, Tokyo, Japan). Biochemical analyses of the blood samples were performed using an autoanalyzer (JCA-RX20; Nihon Denshi, Tokyo, Japan).

Two-step test

The two-step test measures the stride length for evaluation of the walking ability, including muscle strength, balance, and flexibility of the lower limbs [13]. The subjects stood with the toes of both feet behind a starting line and were instructed to take two long steps (as long as possible) and then align both feet. The length of the two steps from the starting line to the tips of the toes at the point where the subject stopped was measured. The two-step test score was calculated as the length of the two steps (cm) ÷ height (cm).

Stand-up test

In this test, the leg strength is assessed by letting the subject stand up on one or both legs from a specified height. The subject stood up from each of the four seats of heights 40, 30, 20, and 10 cm in a descending height order, first with both legs followed by with one leg. A subject who can stand up without leaning back to gain momentum and can maintain the posture for 3 s is considered to have passed that height level [13]. In this study, a subject who was unable to stand up on one leg (right or left) from a 40-cm height was considered to have failed the stand-up test.

The GLFS-25

The GLFS-25 is a self-administered questionnaire consisting of 25 items graded on a 5-point scale, from no impairment (0 points) to severe impairment (4 points) [3]. The sum of the 25 item scores yields a total possible score ranging between 0 and 100, with increasing values indicating increasing LS severity. The validity and reliability of this new measurement have been reported to be satisfactory, with a cutoff score of ≥16 points indicative of LS and with a GLFS-25 score of ≤15 points classified in the non-LS group. For our study, we used the Japanese version of the GLFS-25, ‘‘Locomo 25’’.

LS risk test

The JOA defines the two stages in the LS risk test. LS risk stage 1 is defined as a two-step test score <1.3, difficulty with one-leg standing from a 40-cm-high seat in the stand-up test (either leg), or a 25-question GLFS score ≥7. Subjects meeting any of these criteria were identified to have decline in mobility. LS risk stage 2 is defined as a two-step test score <1.1, difficulty with standing from a 20-cm-high seat using both legs in the stand-up test, or a 25-question GLFS score ≥16. Subjects meeting any of these criteria were diagnosed with progression of functional decline. In this study, the participants that met the criteria for LS risk test stage 1 or 2 were defined as the LS risk group, and the other subjects were normal. To investigate the influence in the age group, a comparison was also performed in the subgroups of participants aged <60 and ≥60 years.

Back muscle and grip strengths

We examined the back muscle strength as the maximum isometric strength of the trunk muscles in a standing position with 30° lumbar flexion by using a digital back muscle strength meter (T.K.K. 5102; Takei Co., Japan) and performing one measurement [14]. We tested the grip strength in a standing position once for each hand by

using a TOEI LIGHT hand grip dynamometer (Toei Light Co., Ltd., Saitama, Japan) [15]. The average value was used to characterize the subject's grip strength.

Timed Up and Go test (TUG)

We measured the time it took a subject to rise from a standard chair (46-cm seat height), walk a distance of 3 m, turn around, walk back to the chair, and sit down [16]. Each subject performed the test twice, both at the maximum pace, and the mean score was used for analyses.

Statistical analysis

Continuous variables are expressed as means [standard deviations (SDs)], and categorical variables are expressed as percentages. Correlations between serum cystatin C level and other variables were analyzed using Spearman's rank correlation coefficients. To investigate the relationship between serum cystatin C level and LS risk, the variables were compared between the normal and LS risk group using the Mann–Whitney *U* test, Fisher's exact test, and generalized linear model (GLM). The GLM analysis was adjusted for age. The threshold value for the predictive value of serum cystatin C level for the presence of LS risk was determined using the receiver operating characteristics (ROC) analysis. All statistical analyses were performed using SPSS Statistics v.24.0 software for Mac (IBM Corp., Armonk, NY, USA). A p-value <0.05 was considered significant in all analyses.

Results

A total of 54 participants were enrolled in this study, and the average age of the subjects was 63.0 years (range: 40–88 years, SDs: 14.3). The participants' demographics, blood test, and physical performance data are listed in Table 1. Percent body fat (PBF) ($p < 0.001$), creatinine ($p < 0.001$), grip strength ($p < 0.001$), back muscle strength ($p < 0.001$), and TUG ($p = 0.028$) showed significant differences between males and females; however, no significant difference was noted in age and serum cystatin C level.

Correlation coefficients of the serum cystatin C level with certain variables are shown in Table 2. The serum cystatin C level had a substantial significant positive correlation with age ($r = 0.671$, $p < 0.001$) and creatinine ($r = 0.609$, $p < 0.001$).

Significant differences were noted in age ($p < 0.001$), serum cystatin C level ($p < 0.001$), creatinine ($p = 0.012$), and TUG ($p = 0.004$) between normal and LS risk groups (Table 3), whereas no significant difference was noted in the grip and back muscle strengths. Furthermore, after controlling for age using GLM analysis, the variables that recognized significant differences were the same.

In the subgroups based on age, no significant difference was found in the serum cystatin C level in the subgroups aged <60 years and without LS risk ($p = 0.22$, Table 4). On the other hand, the serum cystatin C level was significantly high in subjects with LS risk and aged ≥60 years ($p = 0.008$, Table 4).

The ROC curve for the predictive value of the serum cystatin C level for the presence or absence of LS risk had an area under the curve of 0.824 (Figure 1, Table 5). From Youden's index [17], the cutoff value of serum cystatin C level was determined to be 0.685 mg/L for the LS risk group. A serum cystatin C level of ≥0.685 mg/L indicated the presence of LS risk with 79.3% sensitivity and 76.0% specificity (Table 5).

Discussion

This study is the first to investigate the relationship between LS risk and serum cystatin C level, in a prospective large-scale general population. Based on the results of this study, the serum cystatin C level was strongly correlated with LS risk and was significantly higher in LS risk group, even when statistically analyzed with age control. Furthermore, sub-analysis revealed that the serum cystatin C level was significantly higher in the LS risk group aged ≥60 years, reflecting the LS risk in the middle-aged and elderly people.

To date, LS was found to be correlated and associated with various factors [5,18-20]. The LS risk test is a tool for evaluating the possibility of LS development in the future by determining whether leg function is appropriate for the age of the subject. Identification of the risk level as 1 by using the LS risk test may facilitate early recognition of reduced mobility and implementation of preventive measures and appropriate treatment. In particular, the recognition of LS risk level 1 in healthy people—not people who are hospitalized—may enable the introduction of preventive measures at an early stage. Therefore, enrollment of participants who had undergone routine checkup was considered appropriate in this study. However, different factors, such as time, manpower, and equipment used in the determination of the LS risk level, may affect the variables of the LS risk test. For screening purposes, it is important to find early predictors that are objective and easy to use. Therefore, we focused on the serum cystatin C level, which was reported to be not only a good marker of renal function [21-23], but also an early predictor of cardiovascular disease.

The cysteine protease inhibitor cystatin C is a 13-kDa protein consisting of 122 amino acids [24]. Because it is encoded by the housekeeping gene expressed in every nucleated cell, it is produced at a constant rate in the body and secreted extracellularly. Cystatin C does not form a complex with other proteins [25]. It is a basic protein, and under normal physiological conditions, small amounts of catalytically active proteases, released from lysosomes or secreted from infected or dying cells, are effectively blocked by cystatins [26]. A wide-range

scale of biological significance has been advocated for cystatin C, including the regulation of protein catabolism, bone resorption, inflammation, hormone processing, antigen presentation, and T cell-dependent immune response [27–30].

In the recent years, a possible new function of serum cystatin C level as a predictor of the onset and relevance of inflammatory diseases, such as chronic obstructive pulmonary disease and polycystic ovary syndrome, and neoplastic diseases, such as diffuse large B-cell lymphoma, has been determined [30–32]. The serum cystatin C level, including these reports, has received much attention in various diseases and syndromes.

In this study, we evaluated the association between serum cystatin C level and LS risk. In a comparison of the demographic data between normal and LS risk group, significant differences were found in age and serum cystatin C level, but no differences in sex, body mass index (BMI), and PBF. It has been previously reported that age and serum cystatin C level have a positive correlation [33], and this study showed similar results. Generally, the higher the age, the higher the LS risk level. Therefore, in this study, a comparative study was conducted by controlling the age using GLM. Furthermore, participants were divided into those aged <60 and ≥60 years and a sub-analysis was conducted. The serum cystatin C level did not differ significantly between participants aged <60 years regardless of their LS risk, but the serum cystatin C level was significantly higher in those aged ≥60 years with LS risk. In addition, the creatinine was also significantly higher in the LS risk group even when age control was performed. However, creatinine was found to be significantly different between males and females, and since there was no significant difference in those aged ≥60 years, the serum cystatin C level is more significantly associated with LS risk than with creatinine and in particular, may be an early predictor of LS risk in middle-aged and elderly people. If high serum cystatin C level is measured during the medical examination and examination of other diseases, it is necessary to consider the possibility of LS risk. Therefore, it is important

to encourage patients to examine the GLFS-25 and LS risk tests. In this study, TUG, grip strength, and back muscle strength were measured and compared as physical function. In the LS risk, a significant difference was found only in TUG. This result confirmed that reduced mobility and not muscle strength is involved in the LS risk. Further, the ROC analysis in this study confirmed that the serum cystatin C value is a good parameter for LS risk.

There are several limitations of this study. First, the number of participants was relatively small. Second, this research targeted rural residents, which might have affected the LS risk level determination because the living and working environments may differ between rural and urban residents.

In conclusion, the serum cystatin C level is significantly correlated with the LS risk. It is an important marker in the evaluation of early renal function and may be a particularly useful marker reflecting the LS risk in aged ≥60 years, middle-aged, and elderly people. Measurement of serum cystatin C level can be easily performed with little error. Therefore, when the serum cystatin C level is measured at a hospital, it is important to focus on the identification of the LS risk and provide appropriate interventions.

Acknowledgments

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Conflict of interest

None

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Figure legend

Figure 1. The receiver operating characteristic (ROC) curve of serum cystatin C level and LS risk

The area under the ROC curve was 0.824.

Figure 1

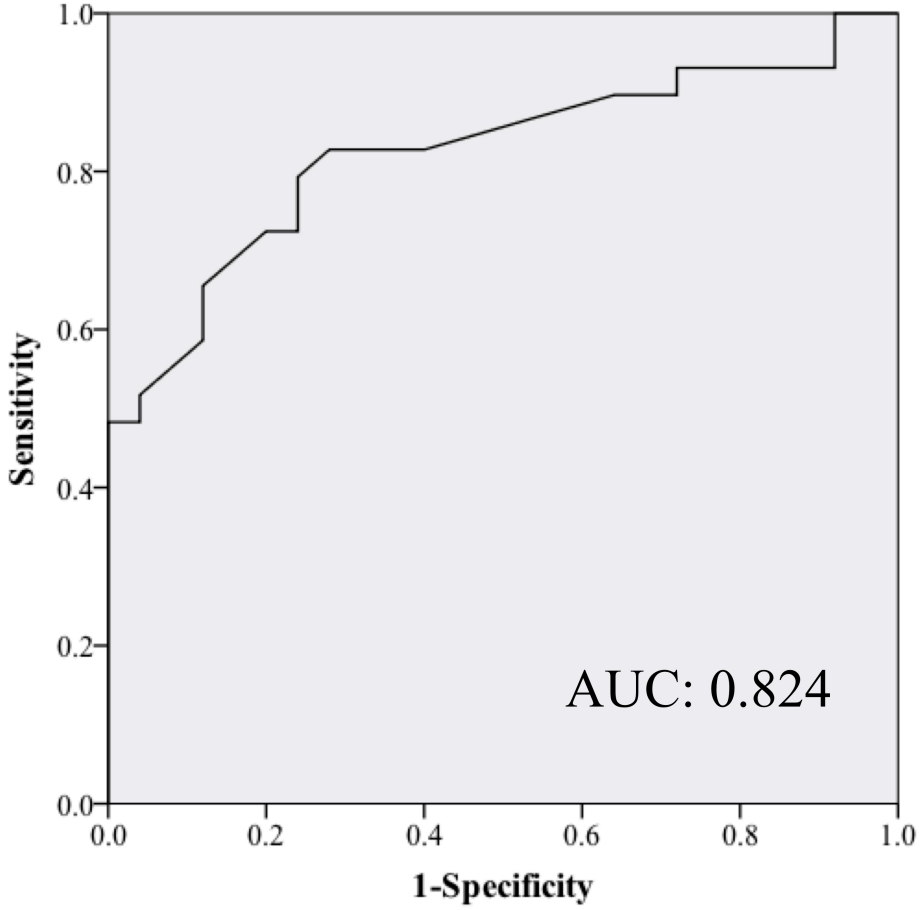
1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1-Specificity
AUC: 0.824

Table 1. Demographic, blood test, physical performance test, and GLFS-25 data of the participants

Variables	**Total**	**Male**	**Female**	**p-value**
Number of participants	54	28	26	
Age (years)	63.0 (14.3)	63.4 (14.0)	62.5 (15.0)	0.85
BMI (kg/m^2)	23.3 (3.5)	24.0 (2.9)	22.6 (3.9)	0.07
PBF (%)	28.0 (7.0)	24.2 (5.1)	31.8 (6.6)	**< 0.001** ***
Serum cystatin C (mg/L)	0.77 (0.19)	0.76 (0.18)	0.77 (0.19)	0.92
Creatinine (mg/dL)	0.75 (0.17)	0.83 (0.15)	0.66 (0.14)	**< 0.001** ***
CRP (mg/dL)	0.09 (0.1)	0.12 (0.2)	0.06 (0.1)	0.10
Grip strength (kg)	27.4 (8.7)	33.8 (6.5)	20.5 (4.3)	**< 0.001** ***
Back muscle strength (kg)	70.0 (28.7)	89.3 (23.4)	47.4 (14.2)	**< 0.001** ***
TUG (s)	6.6 (1.2)	6.2 (0.8)	7.0 (1.4)	**0.028** *
GLFS-25	8.6 (9.7)	6.9 (7.3)	10.5 (11.5)	0.17
Prevalence of LS risk	53.7 %	57.1 %	50.0 %	0.79

Parameter values are shown as mean (standard deviation) or numbers.

BMI, body mass index

PBF, percent body fat

CRP, C-reactive protein

GLFS-25, 25-question geriatric locomotive function scale

TUG, Timed Up and Go

LS, locomotive syndrome

Table 2. Correlation coefficients between certain variables and serum cystatin C level

Variables	**R**	**p-value**
Age (years)	0.671	**< 0.001** ***
Sex	0.013	0.93
BMI (kg/m^2)	-0.022	0.68
PBF (%)	-0.063	0.66
Creatinine (mg/dL)	0.609	**< 0.001** ***
CRP (mg/dL)	0.050	0.72
Grip strength (kg)	-0.049	0.72
Back muscle strength (kg)	-0.102	0.48
TUG (s)	0.219	0.12

***p < 0.001, Spearman's rank correlation coefficient

R, correlation coefficient

BMI, body mass index

PBF, percent body fat

CRP, C-reactive protein

TUG, Timed Up and Go

Table 3. Comparison between normal and LS risk

	Non-adjusted			**Age-adjusted**		
Variables	Normal	LS risk	p-value	Normal	LS risk	p-value
Number of participants	25	29		25	29	
Age (years)	54.7 (11.5)	70.1 (12.7)	**< 0.001** **			
Sex (male/female)	12/13	16/13	0.79	12/13	16/13	0.79
BMI (kg/m^2)	23.7 (4.0)	23.0 (3.0)	0.69	23.9 (0.5)	23.1 (0.4)	0.22
PBF (%)	29.0 (7.8)	27.0 (6.2)	0.21	28.8 (1.4)	26.5 (1.2)	0.23
Serum cystatin C (mg/L)	0.66 (0.07)	0.86 (0.20)	**< 0.001** **	0.67 (0.02)	0.85 (0.02)	**< 0.001** ***
Creatinine (mg/dL)	0.68 (0.13)	0.81 (0.18)	**0.012** *	0.69 (0.03)	0.82 (0.03)	**0.008** **
CRP (mg/dL)	0.10 (0.2)	0.07 (0.1)	0.63	0.12 (0.02)	0.07 (0.02)	0.09
Grip strength (kg)	28.2 (9.4)	26.8 (8.1)	0.79	28.2 (1.9)	27.8 (1.7)	0.89
Back muscle strength (kg)	75.2 (31.1)	64.5 (25.9)	0.20	75.2 (5.9)	66.4 (5.3)	0.29
TUG (s)	6.1 (0.8)	7.0 (1.3)	**0.004** **	6.1 (0.3)	6.9 (0.2)	**0.041** *

*p < 0.05, **p < 0.01, ***p < 0.001, Mann–Whitney *U* test, Fisher's exact test

Parameter values are shown as the mean (standard deviation) or numbers for non-adjusted data and the corrected mean (standard error) or numbers of the mean for age-adjusted data using a generalized linear model.

LS, locomotive syndrome

BMI, body mass index

PBF, percent body fat

CRP, C-reactive protein

TUG, Timed Up and Go

Table 4. Comparison between normal and LS risk in subgroup

	Total			**Aged < 60 years**			**Aged ≥ 60 years**		
Variables	Normal	LS risk	p-value	Normal	LS risk	p-value	Normal	LS risk	p-value
Number of participants	25	29		18	6		7	23	
Age (years)	54.7 (11.5)	70.1 (12.7)	**< 0.001** ***	48.7 (6.5)	50.7 (6.1)	0.58	70.0 (5.3)	75.2 (8.2)	0.14
Sex (male/female)	12/13	16/13	0.79	7/11	6/0	**0.002** **	5/2	10/13	0.39
BMI (kg/m^2)	23.7 (4.0)	23.0 (3.0)	0.69	23.9 (4.3)	24.2 (3.1)	0.72	23.2 (3.4)	22.7 (2.9)	0.70
PBF (%)	29.0 (7.8)	27.0 (6.2)	0.21	30.3 (8.0)	23.5 (5.6)	0.066	25.5 (6.4)	27.9 (6.1)	0.53
Serum cystatin C (mg/L)	0.66 (0.07)	0.86 (0.20)	**< 0.001** ***	0.64 (0.07)	0.67 (0.06)	0.22	0.69 (0.07)	0.91 (0.2)	**0.008** **
Creatinine (mg/dL)	0.68 (0.13)	0.81 (0.18)	**0.012** *	0.67 (0.14)	0.81 (0.12)	0.066	0.71 (0.11)	0.81 (0.20)	0.29
CRP (mg/dL)	0.10 (0.2)	0.07 (0.1)	0.63	0.07 (0.06)	0.05 (0.04)	0.67	0.20 (0.25)	0.08 (0.12)	0.39
Average grip strength (kg)	28.2 (9.4)	26.8 (8.1)	0.79	27.4 (10.0)	34.8 (4.8)	0.066	30.1 (8.0)	24.7 (7.5)	0.13
Back muscle strength (kg)	75.2 (31.1)	64.5 (25.9)	0.20	71.8 (33.6)	92.4 (23.9)	0.19	83.6 (24.1)	58.2 (22.2)	**0.015** *
TUG (s)	6.1 (0.8)	7.0 (1.3)	**0.004** **	6.1 (0.9)	6.2 (0.7)	0.87	6.0 (0.3)	7.2 (1.3)	**0.004** **

*p < 0.05, **p < 0.01, ***p < 0.001, Mann–Whitney *U* test

Parameter values are shown as the mean (standard deviation) or numbers

LS, locomotive syndrome

BMI: body mass index

PBF, percent body fat

CRP, C-reactive protein

TUG, Timed Up and Go

Table 5. The AUC, cutoff value, and sensitivity and specificity of the serum cystatin C level for the presence or absence of LS risk

AUC	SE	p-value	95% CI	Cutoff value	Sensitivity, Specificity(%)
0.824	0.058	0.000	0.711–0.938	0.685	79.3, 76.0

AUC, area under curve

SE, standard error

CI, confidence interval