Received: 18 July 2018 | Revised: 14 August 2018 | Accepted: 14 August 2018

DOI: 10.1002/agm2.12038

ORIGINAL ARTICLE

WILEY Aging Medicine Open Access

Raised serum cystatin C can be a potential biomarker of frailty detected by cumulative deficit model

Venugopalan Gunasekaran[1] | Sharmistha Dey[2] | Avinash Chakrawarty[1] | Prashun Chatterjee[1] | Hem Chandra Sati[3] | Sada Nand Dwivedi[3] | Aparajit Ballav Dey[1]

[1]Department of Geriatric Medicine, All India Institute of Medical Sciences, New Delhi, India

[2]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India

[3]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India

Correspondence
Aparajit Ballav Dey, Department of Geriatric Medicine, All India Institute of Medical Sciences, New Delhi, India.
Email: abdey@hotmail.com

Funding information
This study was done with departmental funding only, and no external funding source was involved.

Abstract

Objective: Identification of frailty by clinical criteria is often delayed to the advanced stage. A reliable biomarker to identify frailty or its risk does not currently exist. We aimed to determine the association between serum cystatin C and frailty in subjects without renal dysfunction.

Methods: We carried out a cross-sectional observational study in the Department of Geriatric Medicine, All India Institute of Medical Sciences, New Delhi, India. The study involved 125 participants, aged 65 years or older. Frailty status was assessed with Frailty Index criteria (cumulative deficit model). Serum cystatin C was estimated with the nephelometry method and its association with frailty was analyzed.

Results: Mean age of the study sample was 76.32 years with 72 (57.6%) male and 53 (42.4%) female participants. Seventy-three subjects were frail; the mean cystatin C levels in the frail and non-frail groups were 1.28 mg/L (±0.39) and 1.12 mg/L (±0.27), respectively, and the difference was significant ($P < 0.05$). A cutoff of 1.12 mg/L was found to be 60.27% sensitive and 57.69% specific in identification of frailty. Multivariate analysis showed that higher cystatin C level was associated with 2.52 (1.05-6.02) times the risk of being frail.

Conclusion: Higher levels of cystatin C were found in frail subjects. Cystatin C seems to be a promising marker for identifying frailty in older adults without renal abnormalities.

KEYWORDS
biomarker, cystatin C, frailty

1 | INTRODUCTION

Frailty is detected clinically by the Frailty Index (FI) as explained in the cumulative deficit model,[1] the frailty phenotype,[2] and so forth. Clinical identification of frailty by any standard scales is only possible after considerable cellular changes have taken place and physical symptoms have manifested. Predicting frailty status before occurrence of clinical symptoms may provide a window of opportunity for early effective intervention. The role of biomarkers comes into play when there is the need for identification of the preclinical stage of frailty or to explain its pathophysiology. No such biomarker is currently available. Various panels of biomarkers have been studied to identify cellular changes in association with frailty and the search continues.[3-7]

The association between kidney function and frailty with serum creatinine was studied in a Cardiovascular Health Study cohort.[2] The

Osteoporosis in Men Study group showed that higher cystatin C was better associated with frailty, using frailty phenotype criteria, than creatinine[8] and was also associated with poorer performance on physical function. This association was present even at normal renal function, independent of several potential confounders and biological mediators. However, this study was done only in a male cohort. Odden et al[9] showed that lifestyle, chronic health conditions, inflammation, and physical inactivity only moderately attenuate the relation between cystatin C and physical function.

Cystatin C is a 122 amino acid, 13-kDa protein belonging to the family of cysteine proteases inhibitors. It is encoded by the CST3 gene, produced by all nucleated cells, and found in all body fluids. Free filtration in glomerulus and maximal reabsorption in proximal tubule of the kidney has made this molecule ideal for calculation of estimated glomerular filtration rate (eGFR)[10] and is better than creatinine-based eGFR estimation.[11] Chronic kidney disease (CKD) estimated by eGFR (cystatin) was associated with higher chances of incident mobility disability and greater decline in gait speed, highlighting the loss of physical independence in elderly CKD patients.[12] Dalrymple et al[13] found that lower eGFR (cystatin) was associated with a higher risk of prevalent and incident frailty, whereas lower eGFR (creatinine) was not. Yaffe et al[14] found that in CKD patients,

TABLE 1 Baseline characteristics of study subjects with mean cystatin C values for frail and non-frail subjects (in mg/L)

Variable	Total number	Frail	Non-frail	Cystatin C frail (mean ± SD)	Cystatin C non-frail (mean ± SD)	*P* value
Overall	125	73	52	1.28 ± 0.39	1.12 ± 0.27	0.012*
Sex						
Male	72	31	41	1.32 ± 0.38	1.15 ± 0.29	0.038*
Female	53	42	11	1.25 ± 0.40	1.00 ± 0.18	0.048*
Mean age (y)	125	76.42 ± 6.44	76.17 ± 5.06			
Age group (y)						
60-74	43	27	16	1.24 ± 0.46	1.00 ± 0.21	0.060
≥75	82	46	36	1.30 ± 0.34	1.17 ± 0.28	0.066
Body mass index (kg/m^2)						
≤23	73	45	28	1.20 ± 0.35	1.11 ± 0.23	0.221
>23	52	28	24	1.40 ± 0.43	1.13 ± 0.32	0.013*
Hypertension						
Yes	59	39	20	1.39 ± 0.42	1.10 ± 0.33	0.008*
No	66	34	32	1.15 ± 0.32	1.13 ± 0.24	0.754
Diabetes						
Yes	19	11	8	1.43 ± 0.49	1.20 ± 0.36	0.272
No	106	62	44	1.25 ± 0.37	1.11 ± 0.26	0.023*
Coronary artery disease						
Yes	17	12	5	1.26 ± 0.37	1.23 ± 0.35	0.895
No	108	61	47	1.29 ± 0.40	1.11 ± 0.27	0.009*
Chronic obstructive lung disease						
Yes	18	13	5	1.45 ± 0.43	1.05 ± 0.26	0.067
No	107	60	47	1.24 ± 0.38	1.13 ± 0.28	0.078
Osteoarthritis knee						
Yes	48	33	40	1.29 ± 0.36	1.22 ± 0.34	0.509
No	77	15	37	1.27 ± 0.42	1.08 ± 0.23	0.016*
Joint pain						
Yes	50	35	15	1.26 ± 0.35	1.19 ± 0.31	0.472
No	75	38	37	1.30 ± 0.43	1.09 ± 0.25	0.147
Cognition						
Normal	89	43	46	1.31 ± 0.44	1.13 ± 0.27	0.022*
Impaired	36	30	6	1.24 ± 0.31	1.06 ± 0.30	0.191
Activities of daily living						
Normal	64	21	43	1.20 ± 0.40	1.10 ± 0.27	0.233
Impaired	61	52	9	1.31 ± 0.39	1.22 ± 0.28	0.507

Values marked with * are statistically significant.

higher cystatin C was associated with poor cognitive function. A study from the United States showed that higher cystatin C level was associated with unsuccessful aging, even when the renal function was normal.[15] In this study, we used cumulative deficit criteria to define frailty to look at the association of cystatin C with frailty in both sexes irrespective of renal function.

2 | METHODS

A cross-sectional observational study was done in the Geriatric Medicine Ward and Outpatient Department, All India Institute of Medical Sciences, New Delhi, India. Institutional ethics committee approval (Ref. No. IESC/T-35/03.01.2014) and subjects' informed written consent were duly taken. A total of 125 study subjects, aged 65 years or older, were included in this study. A convenient sample size, in discussion with a statistician, was used due to logistical issues. Subjects with CKD (Stages 4-5) were excluded as that condition can affect serum cystatin C levels. Consenting individuals were assessed for baseline medical problems and underwent comprehensive geriatric assessment. Frailty status was assessed by the FI criteria (cumulative deficit model).[1] The questionnaire has 36 variables and the number of positive responses was noted. The FI was calculated by dividing the number of positive responses by 36 (total number of variables). The subject is said to be frail when the FI is ≥0.25 (ie, nine or more positive responses). The remaining participants were determined as non-frail. Ten milliliters of venous blood was collected under aseptic precautions and centrifuged at 5000 *g* for 10 minutes to separate serum. Serum was stored in a deep-freeze unit (−80°C), then transported to a laboratory in cold storage where it was thawed and analyzed. Cystatin C was estimated using the nephelometry method: particle-enhanced nephelometric immunoassay (PENIA; Siemens BN-II, Siemens, Erlangen, Germany). The lab reference range was 0.62-1.11 mg/L.

2.1 | Statistical analysis

Statistics was done using STATA (StataCorp., 2011; Stata Statistical Software: Release 12; StataCorp LP, College Station, TX, USA). Descriptive analysis, including absolute frequency distribution, percentage distribution, mean, and standard deviation (SD), were calculated as appropriate. The chi square/Fischer exact test were used to compare qualitative variables and unpaired *t* test/Wilcoxon rank sum test were used to compare quantitative variables. We used one-way analysis of variance to compare between three or more categories. A receiver operating characteristic (ROC) curve was made to identify the cutoff point of cystatin C in the detection of frailty. Based on this cutoff point, the group was divided into two and univariate and multivariate analysis were done for frailty and higher cystatin C with clinically and/or statistically significant variables. The result was considered significant at the 5% level of significance ($P < 0.05$).

3 | RESULTS

The mean age of the study sample was 76.32 years with 72 (57.6%) male and 53 (42.4%) female participants. Seventy-three subjects were frail and 52 were non-frail. Mean cystatin C levels in the frail and non-frail groups were 1.28 mg/L (±0.39) and 1.12 mg/L (±0.27), respectively, and the difference was significant ($P = 0.012$).

Table 1 shows values of cystatin C with other variables. Mean levels of cystatin C in frail male (1.32 ± 0.38 mg/L) and female (1.25 ± 0.40 mg/L) participants were significantly higher than those in non-frail male (1.15 ± 0.29 mg/L) and female (1.00 ± 0.18 mg/L) participants. There was no significant difference between serum cystatin C values when compared between sexes. The mean value of cystatin C increased with age but no significant differences existed between different age groups (<75 and ≥75 years).

The mean cystatin C value was significantly higher in frail subjects with higher body mass index (BMI; >23 kg/m^2) compared to non-frail subjects in the same group. A significant difference was not seen in the BMI ≤23 group. In subjects with hypertension, frail subjects had significantly higher cystatin C levels (1.39 ± 0.42 mg/L) than non-frail subjects (1.10 ± 0.33 mg/L). A significant difference was not seen in the group without hypertension. In subjects with diabetes, coronary

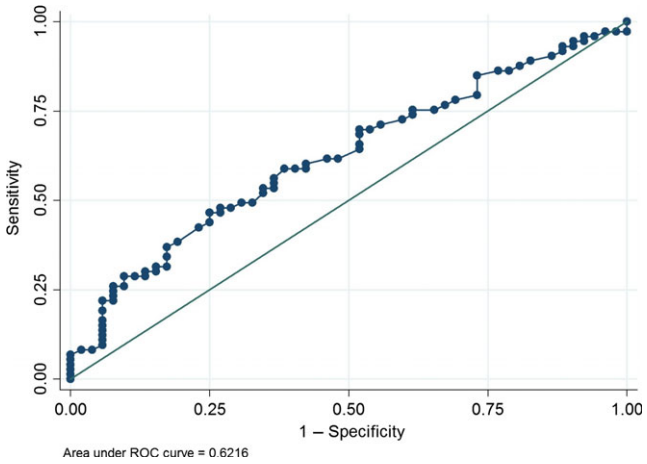

FIGURE 1 Receiver operating characteristic curve, with sensitivity on the *y*-axis and 1-specificity on the *x*-axis, showing serum level of cystatin C with frailty detected by Frailty Index depicted as straight line. Area under the curve was 0.62. The cutoff of 1.12 mg/L is 60.27% sensitive and 57.69% specific for identification of frail older adults

TABLE 2 Multivariate analysis of cystatin C with frailty

Frailty	Unadjusted OR (95% CI)		Adjusted OR[a] (95% CI)	
Cystatin C				
<1.12	1	$P = 0.049$*	1	$P = 0.037$*
≥1.12	2.07 (1.00-4.26)		2.52 (1.05-6.02)	

CI, confidence interval; OR, odds ratio.
[a]Adjusted for age, sex, body mass index, hypertension, and cognition.
Values marked with * are statistically significant.

artery disease, chronic obstructive lung disease, or osteoarthritis of the knee or other joint pain, there was no statistically significant difference between the frail and non-frail groups. In subjects with cognitive impairment, there was a significant difference in the mean cystatin C values of the frail (1.31 ± 0.44 mg/L) and non-frail (1.13 ± 0.27 mg/L) groups. The increase in cystatin C values in frail subjects with impairment of activities of daily living was not significant.

In ROC curve analysis (Figure 1), the cutoff point for cystatin C for identifying frailty was found to be 1.12 mg/L. It had a sensitivity of 60.27% and specificity of 57.69%. An area under the curve of 0.6216 was seen. Based on a cystatin C cutoff of 1.12 mg/L, the study subjects were divided into two groups—lower cystatin C values (<1.12 mg/L) and higher cystatin C values (≥1.12 mg/L)—and these values were compared with frailty status. Subjects with higher cystatin C (≥1.12 mg/L) had 2.01 times (95% confidence interval [CI]: 1.00-4.26) the risk of being frail. This relation persisted even after adjusting for age, sex, BMI, hypertension, and cognitive impairment, where subjects with higher cystatin C had 2.52 times (CI: 1.05-6.02) the risk of being frail (Table 2).

4 | DISCUSSION

In this study group, serum cystatin C was found to be higher in frail subjects. A cutoff point of ≥1.12 mg/L was found to identify frailty with 60% sensitivity and 58% specificity. Based on this cutoff point, the sample subjects were divided into two groups: lower cystatin C values (<1.12 mg/L) and higher cystatin C values (≥1.12 mg/L). Unadjusted analysis showed that subjects with higher cystatin C values were found to have 2.07 times the risk of being frail. Multivariate analysis adjusting for age, sex, BMI, hypertension, and cognitive impairment showed that subjects with higher cystatin C had 2.52 times the increased risk of being frail. Hart et al,[8] of the Osteoporosis in Men Study group, found that higher cystatin C was associated with greater odds of frailty; that study had only male subjects and used frailty phenotype criteria. In our study, subjects from both sexes were included and no significant difference with sex was seen. In subjects with hypertension and cognitive impairment, there was a significant increase of mean cystatin C values in the frail group. In other comorbidities, there was no significant difference in the cystatin C values.

The exact role of cystatin C in the pathophysiology of frailty is not known. Its roles in the inhibition of endogenous or exogenous cysteine peptidases (including muscle cells) and in the modulation of the immune system in aging cells may have links with frailty.[16] However, higher serum cystatin C levels seem to be associated with an increased risk of frailty. This could be tested in larger prospective studies to identify the predictability of incident frailty. A raised serum cystatin C level with other biomarkers may help in the early identification of frailty in older adults during the preclinical stage. The strength of this study was using a newer serum molecule to identify an association with frailty status in older adults. A standardized methodology involving nephelometry was used to determine serum levels of cystatin C. The limitations of the study include the small sample size, the hospital-based population, and the cross-sectional nature of the study. However, this study will help future longitudinal cohort research to study this molecule in relation with frailty.

In summary, raised serum cystatin C was associated with higher risk of being frail. A cystatin C cutoff of 1.12 mg/L was proposed to identify frail subjects with about 60% sensitivity and 58% specificity. Cystatin C has a potential role in identifying frail subjects early and more research is needed.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Venugopalan Gunasekaran http://orcid.org/0000-0002-9379-2267

REFERENCES

1. Song X, Mitnitski A, Rockwood K. Prevalence and 10-year outcomes of frailty in older adults in relation to deficit accumulation. *J Am Geriatr Soc*. 2010;58:681-687.
2. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56:M146-M157.
3. Cesari M, Penninx BWJH, Pahor M, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci*. 2004;59:242-248.
4. Leng SX, Xue Q-L, Tian J, Walston JD, Fried LP. Inflammation and frailty in older women. *J Am Geriatr Soc*. 2007;55:864-871.
5. Kumar R, Mohan N, Upadhyay AD, et al. Identification of serum sirtuins as novel noninvasive protein markers for frailty. *Aging Cell*. 2014;13:975-980.
6. Mitnitski A, Collerton J, Martin-Ruiz C, et al. Age-related frailty and its association with biological markers of ageing. *BMC Med*. 2015;13:161.
7. Lippi G, Jansen-Duerr P, Viña J, et al. Laboratory biomarkers and frailty: presentation of the FRAILOMIC Initiative. *Clin Chem Lab Med*. 2015;53:e253-e255.
8. Hart A, Paudel ML, Taylor BC, et al. Cystatin C and frailty in older men. *J Am Geriatr Soc*. 2013;61:1530-1536.
9. Odden MC, Chertow GM, Fried LF, et al. Cystatin C and measures of physical function in elderly adults: the Health, Aging, and Body Composition (HABC) Study. *Am J Epidemiol*. 2006;164:1180-1189.
10. Chew JSC, Saleem M, Florkowski CM, George PM. Cystatin C: a paradigm of evidence based laboratory medicine. *Clin Biochem Rev*. 2008;29:47-62.
11. Roos JF, Doust J, Tett SE, Kirkpatrick CMJ. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children: a meta-analysis. *Clin Biochem*. 2007;40:383-391.
12. Liu CK, Lyass A, Massaro JM, D'Agostino RB, Fox CS, Murabito JM. Chronic kidney disease defined by cystatin C predicts mobility disability and changes in gait speed: the Framingham Offspring Study. *J Gerontol A Biol Sci Med Sci*. 2014;69:301-307.
13. Dalrymple LS, Katz R, Rifkin DE, et al. Kidney function and prevalent and incident frailty. *Clin J Am Soc Nephrol*. 2013;8:2091-2099.
14. Yaffe K, Kurella-Tamura M, Ackerson L, et al. Higher levels of cystatin C are associated with worse cognitive function in older

adults with chronic kidney disease: the Chronic Renal Insufficiency Cohort Cognitive Study. *J Am Geriatr Soc*. 2014;62:1623-1629.
15. Sarnak MJ, Katz R, Fried LF, et al. Cystatin C and aging success. *Arch Intern Med*. 2008;168:147-153.
16. Mussap M, Plebani M. Biochemistry and clinical role of human cystatin C. *Crit Rev Clin Lab Sci*. 2004;41:467-550.

How to cite this article: Gunasekaran V, Dey S, Chakrawarty A, et al. Raised serum cystatin C can be a potential biomarker of frailty detected by cumulative deficit model. *Aging Med*. 2018;1:149–153. https://doi.org/10.1002/agm2.12038