Association of cathepsin B and cystatin C with an age-related pulmonary subclinical state in a healthy Chinese population

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Nan Wang, Yajun Yuan, Xiaojuan Bai, Wen Han, Lulu Han and Bijuan Qing

Abstract

Background: Cathepsin B (CTSB) and cystatin C (CYSC) are new biomarkers for several physiological and pathological processes as their activities increase with age. The aim of this study was to explore population-level associations between serum CTSB and CYSC with an age-related pulmonary subclinical state.

Methods: We examined 401 healthy participants (aged 36–87 years, of which 44.3% were male) in northern Chinese cities. We used a standard spirometer to determine lung function. Serum CTSB and CYSC levels were measured by enzyme-linked immunosorbent assay (ELISA).

Results: For all participants, serum CTSB was related to maximum vital capacity (VC MAX), forced vital capacity (FVC), forced expiratory volume in 1 s, peak expiratory flow, forced expiratory flow at 25% of FVC, forced expiratory volume in 3 s (FEV3), and inspiratory vital capacity (VC IN). These associations were lost after full adjustment. CYSC remained significantly associated with inspiratory capacity (IC), breath frequency (BF; $p < 0.001$), minute ventilation (MV), the ratio of FEV3 and FVC (FEV3%FVC), and expiratory reserve volume ($p < 0.05$) after adjusting for all other possible confounders. In males, serum CYSC levels exhibited significant and independent associations with FVC, FEV3 ($p < 0.05$), and IC ($p < 0.001$) and serum CTSB levels exhibited significant and independent associations with BF ($p < 0.05$).

Conclusions: Our results confirmed serum CYSC concentration associations with an age-related lung function in healthy people. However, the association between serum CTSB and lung function was not well confirmed. Serum measurements of CYSC may provide valuable predictors of pulmonary function in healthy people, especially healthy elderly adults.

The reviews of this paper are available via the supplemental material section.

Keywords: cathepsin B, cystatin C, elderly population, lung function tests, pulmonary subclinical state

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Correspondence to:
Nan Wang
Department of Gerontology and Geriatrics, The Fifth Affiliated Hospital of Sun Yat-sen University, Meihua East Road, Zhuhai, Guangdong 519000, China
wangnanyo@163.com

Xiaojuan Bai
Department of Gerontology and Geriatrics, Shengjing Hospital of China Medical University, Shenyang, 110000, Liaoning, China
yuanyj238@163.com

Yajun Yuan
Department of Interventional Medicine, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China

Wen Han
Lulu Han
Department of Gerontology and Geriatrics, Shengjing Hospital of China Medical University, Shenyang, China

Bijuan Qing
Department of Gerontology and Geriatrics, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China

Introduction

Aging is a complex process involving numerous cellular and molecular changes that contribute to age-related multiple organ dysfunction.[1–3] At the cellular level, cells undergo the development of senescent phenotypes, which are responsible for the expression of multiple phenotypes in aging.[4–6] While numerous studies have focused on the cellular and molecular basis of these age-mediated changes in multiple organ structures and functions, the exact mechanisms are not well understood.[5–7]

Thanks to aging, cells processing cytosolic changes result in physiologically acidic environments inside the cell, the lysosomal membrane becomes permeable to lysosomal enzymes, which have important roles in age-related pathologic processes.[8] Cathepsin B (CTSB), a lysosomal proteolytic enzyme, is closely associated with pathological processes in several diseases.[9–14] Under certain conditions, such as cell damage caused by aging, CTSB is released from lysosomes and is accompanied by increased activity in serum.[9] The extracellular activity of CTSB

leads to the degradation of matrix components. This extracellular activity is regulated specifically by its natural inhibitor, cystatin C (CYSC), a member of the cystatin superfamily.[14–16] Imbalances between CTSB and CYSC regulation may be crucial in the breakdown of extracellular matrix (ECM) components, contributing to pathologies such as tumor metastasis, atherosclerosis, arthritis, nephropathy, pancreatitis, and inflammatory lung disease.[15–19] Our previous study confirmed that serum CTSB levels increased with age and were correlated with an age-related cardiovascular-renal subclinical state in healthy Chinese populations.[20] However, little research has investigated associations between serum CTSB concentrations and declines in other normal, age-related organ functions, particularly in the respiratory system. This study proposes that CTSB and CYSC could play crucial roles in myofibrogenesis and the progression of lung fibrosis. CTSB and CYSC are secreted into the pericellular environment or are associated with cell surfaces and nuclei.[21] The study of human aging is difficult in terms of separating the aging process from concomitant diseases, or defining normality and abnormality in the aging process. Aging is associated with structural and functional changes, as evidenced by variations in parameters that are modified during age-related disease, and have been proposed as early markers of disease.[7] In this study, we evaluated several parameters of age-related pulmonary changes and explored their associations with serum CTSB and CYSC levels. We wanted to assess the potential contribution of CTSB and CYSC to the pathophysiology of an age-related pulmonary subclinical state in healthy populations. With the elderly population growing, elucidating the cellular and molecular mechanisms behind aging is critical in establishing appropriate and measured strategies to prevent aging-related disease and promote healthy aging.

Methods

Study participants

Participants for this community-based cross-sectional study were recruited from 15 different communities in northern China cities during 2011. All participants were selected based on results from a physician's questionnaire and a clinical biochemical examination. Inclusion criteria included the absence of mental disorders and the presence of good mental health, self-care ability, and normal social interactions and adaptability. Exclusion criteria included participants with hypertension, diabetes, and coronary heart disease, and those with preexisting cardio-respiratory diseases, such as chronic obstructive pulmonary disease (COPD), asthma, and obvious spinal deformities. Those previously or currently undergoing treatment for pulmonary tuberculosis, or upper and lower respiratory tract infections were excluded. A total of 1500 participants were randomly selected. Of these, 999 subjects declined to sign the informed consent or had pre-existing conditions as well as chronic infections or tumors, or taking medication that altered lung function, and were excluded from the study. The remaining 501 healthy subjects who signed the informed consent underwent the following testing: fasting blood glucose (FBG), serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), serum creatinine (SCr), CYSC, pulmonary function, electrocardiogram, echocardiography, and carotid artery ultrasonography. After excluding individuals for aberrant physical examinations or laboratory results, 401 qualified healthy participants (178 men and 223 women) remained. This study was approved by Medical Ethics Committee of General Hospital of People's Liberation Army and all participants provided written informed consent.

Clinical methods and laboratory tests

All participants underwent a clinical examination and completed a detailed questionnaire at enrollment. Basic data including age, sex, height, and weight were recorded. All weights were measured to the nearest 0.1 kg using standard weighing scales. All heights were measured to the nearest 0.1 cm using a standard measuring stick, without shoes, with the feet together, standing with the eyes looking straight ahead. Height was converted to meters and body mass index (BMI) was calculated from weight (kg) and height (m^2) and expressed as kg/m^2. Blood pressure was measured after a 10-min rest. Venous blood was collected 12 h after fasting commenced; some blood samples were used for routine biochemical tests to determine fasting levels of FBG, TG, TC, HDL-C, and LDL-C. The remaining blood samples were centrifuged and stored (−80°C). Serum CTSB and CYSC levels were measured using

ELISA (USCNK, Wuhan, China), according to the manufacturer's instructions.

Pulmonary function tests

A standard Spirometer (Micro lab ML3500 MK8, Cardinal Health, Germany 234 GMBH, Hoechberg, Germany) with a disposable mouthpiece was used. The ambient temperature, barometric pressure, and time of measurement were all recorded. Participants were given instructions and then a personal demonstration on how to use the spirometer. Participants were relaxed, with dentures removed and tight-fitting clothes loosened. After measuring the basal respiratory rate, individuals were told to sit upright in a straight-backed chair, with their belt loosened. The participant was then asked to breathe normally for approximately 20 s, then breathe in as hard as possible and hold the breath. The lips were applied firmly around the mouthpiece of the spirometer and the participant told to breathe out as quickly and as forcibly as possible into the spirometer. The mouthpiece was checked for leakages. The procedure was repeated if leakages were observed. The equipment automatically selects the best of three measurements, when the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines have been met (three good blows within 5% or 0.151 (150 ml). Lung functions were recorded as percentages, medians [interquartile ranges (IQR)], minimum and maximums, while sociodemographic data were recorded as mean [standard deviation (SD)].

Statistical analysis

The Kolmogorov–Smirnov method was used for normality testing. The data were expressed as mean ± SD. One-way ANOVA was used for comparisons between groups. The least-significant difference (LSD) method or Dunnett's T3 was used for multiple comparisons of the means of each group, according to homogeneity of the variance. Categorical data were expressed as numbers, and differences in variables among the groups were examined using the chi-square test and Fisher probabilities. Spearman correlation coefficients were used for bivariate correlation analyses. Multiple regression analyses were used to adjust for possible confounding variables. All p-values < 0.05 indicated statistical significance. All statistical analyses were performed using SPSS 19.0 statistical software.

Results

Basic characteristics of participants based on age and gender

Table 1 presents basic study characteristics according to age and gender. All variables except for diastolic blood pressure (DBP), white blood cells (WBC), red blood cells (RBC), HDL, and tidal volume (VT) were significantly different between the two age groups ($p < 0.05$). CTSB and CYSC levels increased significantly with age ($p < 0.05$); however, pulmonary variables, except MV and BF, decreased with age ($p < 0.05$). There were gender differences for all variables except DBP, albuminous (ALB), FEV1%FVC, FEV1%VC MAX, and FEV3%FVC in the same age group. Women had significantly lower levels of CTSB, CYSC, VC MAX, FVC, FEV1, PEF, FEF25, forced expiratory flow at 50% of FVC (FEF50), forced expiratory flow at 75% of FVC (FEF75), maximal mid-expiratory flow (MMEF75/25), IC, MV, FEV3, VT, ERV, VC IN, and higher levels of FEV1%FVC, FEV1%VC MAX, and BF (all $p < 0.05$).

Correlation of serum CTSB and CYSC levels with basic characteristics and age-related pulmonary parameters

Correlations between CTSB and CYSC levels and clinical and laboratory parameters were established (Tables 2 and 3). Spearman's rank correlation is used for bivariate correlation analyses. Usually, the correlation coefficient, r, was interpreted as follows: $r < 0.2$ indicated meaningless correlation, $0.2 < r < 0.4$ indicated weak correlation, $0.4 < r < 0.6$ indicated moderate correlation, $0.6 < r < 0.8$ indicated strong correlation, and $0.8 < r$ indicated very strong correlation.

In all participants, there are weak correlation between serum CTSB and age, height, weight, smoking, RBC, HGB, HDL, CYSC, and FEV3 ($0.2 < r < 0.4$, $p < 0.05$). In males, serum CTSB was positively correlated with WBC, CYSC, and BF, and negatively correlated with ALB ($0.2 < r < 0.4$, $p < 0.05$). In females, serum CTSB was positively correlated with age, SBP, and CYSC ($0.2 < r < 0.4$, $p < 0.05$).

In all participants, serum CYSC was correlated with age ($r = 0.535$, $p < 0.001$) and BMI, HDL, CTSB, VC MAX, FVC, FEV 1, FEV1%VC MAX, FEF25, FEF50, FEF75, MMEF75/25,

Table 1. Basic characteristics of subjects based on age group and gender.

	⩽60 years			>60 years		
	Total (*n* = 217)	**Male (*n* = 90)**	**Female (*n* = 127)**	**Total (*n* = 184)**	**Male (*n* = 88)**	**Female (*n* = 96)**
Age (years)	49.59 ± 7.13	50.09 ± 7.13	49.23 ± 7.14	70.81 ± 7.13**	71.03 ± 7.10**	70.63 ± 7.19**
Height (cm)	165.16 ± 7.49	171.34 ± 5.34	160.86 ± 5.51##	162.88 ± 7.80**	169.29 ± 4.40**	157.65 ± 5.81**##
Weight (cm)	64.95 ± 11.58	73.35 ± 11.10	59.11 ± 7.67##	63.30 ± 10.60**	68.66 ± 9.51**	58.96 ± 9.43##
BMI (kg/m^2)	23.71 ± 3.18	24.96 ± 3.45	22.83 ± 2.66##	23.82 ± 3.19	23.92 ± 2.88*	23.73 ± 3.43*
Cigarette smoking						
Never	155 (164.9)	31 (43.0)	123 (121.8)	147 (137.1)	52 (40.0)	92 (93.2)
Former	9 (7.1)	9 (6.7)	1 (2.3)	4 (5.9)	4 (6.3)	3 (1.7)
Current	49 (41.0)	45 (35.2)	4 (4.0)	26 (34.0)	23 (32.8)	3 (3.0)
SBP (mmHg)	126.88 ± 17.82	127.48 ± 17.61	126.47 ± 18.02	136.61 ± 20.41**	132.18 ± 16.96	138.16 ± 22.24**##
DBP (mmHg)	77.31 ± 10.73	78.23 ± 11.35	76.69 ± 10.28	78.89 ± 9.96	78.58 ± 9.94	79.15 ± 10.02
WBC (10^9/L)	5.89 ± 1.35	6.31 ± 1.54	5.60 ± 1.13##	5.91 ± 1.45	6.00 ± 1.56	5.84 ± 1.36
RBC (10^12/L)	4.60 ± 0.43	4.94 ± 0.36	4.37 ± 0.31##	4.50 ± 0.43*	4.72 ± 0.41**	4.32 ± 0.35##
HGB (g/L)	138.13 ± 15.68	151.94 ± 11.03	128.78 ± 10.63##	137.02 ± 12.87	145.29 ± 11.87**	130.14 ± 9.08##
ALB (g/L)	48.43 ± 2.61	48.81 ± 2.35	48.18 ± 2.76	47.34 ± 2.62**	47.03 ± 2.74**	47.60 ± 2.49
TG (mmol/L)	1.33 ± 0.72	1.58 ± 0.64	1.17 ± 0.83#	1.21 ± 0.64	1.05 ± 0.42**	1.35 ± 0.70##
TC (mmol/L)	5.08 ± 0.92	4.95 ± 0.85	5.16 ± 0.96	5.22 ± 1.01	4.90 ± 0.85	5.47 ± 1.06*##
HDL (mmol/L)	1.44 ± 0.35	1.30 ± 0.30	1.53 ± 0.34##	1.46 ± 0.36	1.34 ± 0.32	1.55 ± 0.37##
LDL (mmol/L)	3.18 ± 0.78	3.13 ± 0.70	3.22 ± 0.83	3.38 ± 0.93*	3.18 ± 0.81	3.55 ± 0.99**##
FBG (mmol/L)	5.24 ± 0.69	5.37 ± 0.78	5.15 ± 0.60#	5.39 ± 0.85	5.37 ± 0.57	5.40 ± 1.03*
CTSB (ng/ml)	1.15 ± 0.68	1.51 ± 0.71	0.91 ± 0.55##	1.48 ± 0.82**	1.77 ± 0.86*	1.25 ± 0.71**##
CYSC (mg/L)	0.77 ± 0.14	0.84 ± 0.13	0.73 ± 0.13##	0.98 ± 0.27**	1.00 ± 0.24**	0.97 ± 0.29**
VC MAX	3.44 ± 0.74	4.03 ± 0.66	3.03 ± 0.48##	2.75 ± 0.70**	3.20 ± 0.65**	2.38 ± 0.49**##
FVC	3.35 ± 0.73	3.94 ± 0.62	2.94 ± 0.47##	2.66 ± 0.69**	3.12 ± 0.64**	2.29 ± 0.47**##
FEV 1	2.77 ± 0.59	3.24 ± 0.55	2.46 ± 0.38##	2.14 ± 0.61**	2.48 ± 0.61**	1.86 ± 0.46**##
FEV1%FVC	83.23 ± 6.71	82.21 ± 7.28	83.93 ± 6.22	79.98 ± 9.17**	78.96 ± 8.39**	80.81 ± 9.71**
FEV1%VC MAX	80.88 ± 6.42	80.36 ± 6.95	81.24 ± 6.03	77.28 ± 9.08**	76.99 ± 8.35**	77.51 ± 9.66**
PEF	6.70 ± 1.84	8.14 ± 1.69	5.71 ± 1.17##	5.42 ± 2.01**	6.61 ± 2.15**	4.45 ± 1.42**##
FEF25	6.07 ± 1.73	7.20 ± 1.78	5.29 ± 1.18##	4.76 ± 1.87**	5.62 ± 1.97**	4.06 ± 1.47**##

(Continued)

Table 1. (Continued)

	⩽60 years			>60 years		
	Total (*n* = 217)	**Male (*n* = 90)**	**Female (*n* = 127)**	**Total (*n* = 184)**	**Male (*n* = 88)**	**Female (*n* = 96)**
FEF50	3.51 ± 1.16	4.09 ± 1.30	3.11 ± 0.85##	2.59 ± 1.09**	2.92 ± 1.19**	2.33 ± 0.93**##
FEF75	1.17 ± 0.44	1.33 ± 0.48	1.06 ± 0.38##	0.78 ± 0.42**	0.88 ± 0.47**	0.71 ± 0.36**##
MMEF75/25	2.77 ± 0.89	3.23 ± 0.95	2.45 ± 0.69##	1.93 ± 0.87**	2.20 ± 0.94**	1.72 ± 0.75**##
IC	2.30 ± 0.52	2.60 ± 0.53	2.11 ± 0.40##	1.99 ± 0.58**	2.23 ± 0.61**	1.76 ± 0.43**##
MV	10.45 ± 4.87	11.42 ± 6.05	9.81 ± 3.78#	11.65 ± 5.76*	12.42 ± 5.82	10.96 ± 5.65
FEV3	3.23 ± 0.69	3.68 ± 0.60	2.79 ± 0.44##	2.54 ± 0.71**	2.87 ± 0.66**	2.15 ± 0.55**##
FEV3%FVC	95.41 ± 3.63	94.98 ± 3.99	95.82 ± 3.23	94.10 ± 5.89	94.59 ± 4.71	93.51 ± 7.08*
VT	0.63 ± 0.28	0.72 ± 0.33	0.57 ± 0.23##	0.64 ± 0.32	0.74 ± 0.34	0.55 ± 0.28##
BF	17.67 ± 6.13	16.78 ± 6.62	18.26 ± 5.73	19.48 ± 7.32*	17.82 ± 5.85	20.97 ± 8.18**##
ERV	1.14 ± 0.57	1.44 ± 0.64	0.95 ± 0.43##	0.84 ± 0.51**	0.99 ± 0.59**	0.70 ± 0.35**##
VC IN	3.03 ± 0.76	3.59 ± 0.72	2.65 ± 0.51##	2.44 ± 0.69**	2.83 ± 0.69**	2.12 ± 0.51**##

Normally distributed data were expressed as the mean ± SD, with differences examined using independent-sample *t*-tests. Data not normally distributed were shown as the median and IQR. Comparisons of these variables among groups were performed by the Mann–Whitney *U* Test. *p*-values are the outcome of the analysis of the independent-sample *t*-tests or Mann-Whitney *U* Test. Categorical data were expressed as numbers and the differences in the variables among the groups were examined using the chi-square test and Fisher probabilities.
*Significant difference ($p < 0.05$).
**Significant difference ($p < 0.01$) when compared with ⩽60 years group, respectively.
#Significant difference ($p < 0.05$).
##Significant difference ($p < 0.01$) when compared with the males in the same age group, respectively.
ALB, albuminous; BF, breath frequency; BMI, body mass index; CTSB, cathepsin B; CYSC, cystatin C; DBP, diastolic blood pressure; ERV, expiratory reserve volume; FBG, fasting blood glucose; FEF25, forced expiratory flow at 25% of FVC; FEF50, forced expiratory flow at 50% of FVC; FEF75, forced expiratory flow at 75% of FVC; FEV 1, forced expiratory volume in 1 s; FEV1%FVC, the ratio of FEV1 and FVC; FEV1%VC, the ratio of FEV1 and vital capacity; FEV3%FVC, forced expiratory volume in 3 s/FVC; FEV3, Forced expiratory volume in three seconds; FVC, forced vital capacity; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IC, inspiratory capacity; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MMEF75/25, maximal mid-expiratory flow; MV, minute ventilation; PEF, peak expiratory flow; RBC, red blood cells; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VC IN, inspiratory vital capacity; VC MAX, maximum vital capacity; VT, tidal volume; WBC, white blood cells.

MV, FEV3, and FEV3%FVC ($0.2 < r < 0.4$, $p < 0.05$). In males, serum CYSC was correlated with age, VC MAX, FVC, FEV 1, PEF, FEV3 ($0.4 < r < 0.6$, $p < 0.001$), and RBC, HGB, ALB, CTSB, FEV1%VC MAX, FEF25, FEF50, FEF75, MMEF75/25, IC, and VC IN ($0.2 < r < 0.4$, $p < 0.05$). In females, serum CYSC was correlated with age ($r = 0.653$, $p < 0.001$) and VC MAX, FVC, FEV 1, MMEF75/25, FEV3 ($0.4 < r < 0.6$, $p < 0.001$), and height, BMI, SBP, TG, CTSB, FEV1%VC MAX, PEF, FEF25, FEF50, FEF75, IC, FEV3%FVC, BF, ERV, and VC IN ($0.2 < r < 0.4$, $p < 0.05$).

The relationship between serum CTSB and CYSC levels and pulmonary parameters using a stepwise multiple regression model

Pulmonary parameters, independently associated with serum CTSB levels, were examined using a multiple regression model, using a stepwise entry (Table 4). Independent predictors of serum CTSB levels included in the model were parameters correlated with CTSB levels in the pairwise correlation analysis, and the traditional pulmonary risk factors. For all participants, the data showed that serum CTSB levels were significantly associated with VC MAX, FVC, FEV 1, PEF, FEF25, MV, FEV3, and VC IN (all $p < 0.05$),

Table 2. Correlation of serum CTSB levels with basic characteristics and pulmonary parameters using R_p (Spearman's correlation coefficient).

	CTSB					
	Total		Male		Female	
	r_p	p	r_p	p	r_p	p
Age (years)	0.211**	0.000	0.169*	0.032	0.226**	0.001
Height (cm)	0.263**	0.000	0.115	0.143	−0.111	0.098
Weight (cm)	0.215**	0.000	0.111	0.157	−0.062	0.353
BMI (kg/m^2)	0.100*	0.049	0.078	0.323	−0.018	0.793
Cigarette smoking	0.220**	0.000	0.018	0.827	0.072	0.283
SBP (mmHg)	0.113*	0.025	0.051	0.521	0.220**	0.001
DBP (mmHg)	0.051	0.319	−0.001	0.986	0.075	0.263
WBC	0.151**	0.003	0.213**	0.007	0.016	0.814
RBC	0.219**	0.000	0.030	0.702	0.036	0.595
HGB	0.259**	0.000	−0.008	0.923	−0.003	0.959
ALB	−0.065	0.206	−0.202**	0.010	0.008	0.910
TG (mmol/L)	0.068	0.184	0.064	0.421	0.077	0.250
TC (mmol/L)	−0.068	0.186	0.000	0.999	0.013	0.848
HDL (mmol/L)	−0.201**	0.000	−0.115	0.144	−0.054	0.422
LDL (mmol/L)	−0.023	0.647	0.027	0.730	0.018	0.790
FBG (mmol/L)	0.049	0.342	−0.035	0.658	0.064	0.342
CYSC (mg/L)	0.332**	0.000	0.235**	0.006	0.287**	0.000
VC MAX	0.183**	0.001	−0.077	0.352	−0.078	0.267
FVC	0.185**	0.000	−0.089	0.285	−0.084	0.233
FEV 1	0.151**	0.005	−0.101	0.222	−0.076	0.276
FEV1%FVC	−0.098	0.055	−0.054	0.493	−0.050	0.458
FEV1%VC MAX	−0.079	0.120	−0.104	0.186	−0.026	0.695
PEF	0.194**	0.007	−0.015	0.854	−0.066	0.348
FEF25	0.147**	0.006	−0.051	0.539	−0.075	0.287
FEF50	0.006	0.913	−0.073	0.357	−0.159*	0.017
FEF75	−0.014	0.782	−0.140	0.074	−0.077	0.250
MMEF75/25	0.009	0.863	−0.088	0.265	−0.133*	0.047
IC	0.136*	0.016	−0.055	0.525	−0.029	0.697

(Continued)

Table 2. (Continued)

	CTSB					
	Total		Male		Female	
	r_p	*p*	r_p	*p*	r_p	*p*
MV	0.119*	0.025	0.113	0.164	0.039	0.582
FEV3	0.245**	0.002	−0.077	0.492	−0.023	0.839
FEV3%FVC	−0.084	0.259	−0.132	0.205	−0.076	0.477
VT	0.093	0.079	−0.017	0.835	−0.001	0.987
BF	0.042	0.433	0.209**	0.010	0.033	0.645
ERV	0.077	0.155	−0.057	0.488	−0.036	0.613
VC IN	0.142**	0.008	−0.074	0.370	−0.142*	0.043

ALB, albuminous; BF, breath frequency; BMI, body mass index; CTSB, cathepsin B; CYSC, cystatin C; DBP, diastolic blood pressure; ERV, expiratory reserve volume; FBG, fasting blood glucose; FEF25, forced expiratory flow at 25% of FVC; FEF50, forced expiratory flow at 50% of FVC; FEF75, forced expiratory flow at 75% of FVC; FEV 1, forced expiratory volume in 1 s; FEV1%FVC, the ratio of FEV1 and FVC; FEV1%VC, the ratio of FEV1 and vital capacity; FEV3%FVC, forced expiratory volume in 3 s/FVC; FEV3, Forced expiratory volume in three seconds; FVC, forced vital capacity; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IC, inspiratory capacity; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MMEF75/25, maximal mid-expiratory flow; MV, minute ventilation; PEF, peak expiratory flow; RBC, red blood cells; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VC IN, inspiratory vital capacity; VC MAX, maximum vital capacity; VT, tidal volume; WBC, white blood cells.
p values are from analysis of variance.
*Significant difference ($p < 0.05$).
**Significant difference ($p < 0.01$).

and these associations were lost after full adjustment. In males, serum CTSB levels exhibited significant and independent association with BF ($p < 0.05$).

After adjusting for all other possible confounders for all participants, CYSC remained significantly associated with IC ($p < 0.001$), MV ($p = 0.002$), FEV3%FVC ($p = 0.013$), BF ($p < 0.001$), and ERV ($p < 0.05$). In males, serum CYSC levels exhibited significant and independent associations with FVC ($p < 0.05$), IC ($p = 0.001$) and FEV3 ($p = 0.008$) (Table 5).

Discussion

In the present study, we report, for the first time, the association of serum CTSB and CYSC concentration with age-related pulmonary functions in healthy people. We selected participants from a Chinese population free of hypertension, diabetes, coronary heart disease, and other acute and chronic diseases, thus excluding many potential disease confounders.

To our knowledge, this is the first study to analyze correlations between serum CYSC levels and declines in normal, age-related pulmonary functions in healthy people. We found that serum CYSC levels correlated with an age-related pulmonary subclinical state. However, the association between serum CTSB and lung function was not well confirmed. CTSB is a lysosomal enzyme that plays roles in many physiological and pathological processes.[9–16] Its expression has been demonstrated in several organs and tissues, especially macrophages, hepatocytes, renal tubules, epithelium, and fibroblasts.[11,22,23] CYSC is the main inhibitor of CTSB, which is tightly regulated by CYSC in ECM remodeling.[14–16] Imbalances between CTSB and CYSC are implicated in glomerulosclerosis, chronic inflammatory lung disease, cardiomyopathy, and atherosclerotic lesions with senescence-associated phenotypes.[15–19] Moreover, CTSB is of special interest as a risk factor in age-related dysfunction, as its activity increases with age and it is important in oxidative stress, autophagy, inflammation, and apoptosis processes.[24–26]

Table 3. Correlation of serum CYSC levels with basic characteristics and pulmonary parameters using R_p (Spearman's correlation coefficient).

	CYSC					
	Total		Male		Female	
	r_p	p	r_p	p	r_p	p
Age (years)	0.535**	0.000	0.526**	0.000	0.653**	0.000
Height (cm)	−0.058	0.308	0.017	0.843	−0.280**	0.000
Weight (cm)	−0.092	0.106	0.054	0.529	0.095	0.178
BMI (kg/m^2)	0.250**	0.000	0.030	0.727	0.256**	0.000
Cigarette smoking	0.126*	0.021	−0.029	0.741	0.041	0.568
SBP (mmHg)	0.139*	0.015	0.130	0.128	0.269**	0.000
DBP (mmHg)	0.038	0.509	0.026	0.765	0.101	0.153
WBC	0.056	0.333	0.002	0.984	0.070	0.327
RBC	−0.044	0.443	−0.207*	0.015	−0.010	0.889
HGB	−0.040	0.493	−0.225**	0.008	0.067	0.354
ALB	−0.168**	0.003	−0.309**	0.000	−0.137	0.054
TG (mmol/L)	0.180**	0.002	−0.025	0.775	0.307**	0.000
TC (mmol/L)	−0.064	0.266	−0.166	0.052	0.095	0.184
HDL (mmol/L)	−0.272**	0.000	−0.175*	0.041	−0.151*	0.034
LDL (mmol/L)	0.022	0.696	−0.100	0.242	0.146*	0.040
FBG (mmol/L)	−0.026	0.651	−0.150	0.078	0.045	0.533
CTSB (mg/L)	0.332**	0.000	0.235**	0.006	0.287**	0.000
VC MAX	−0.212**	0.000	−0.408**	0.000	−0.489**	0.000
FVC	−0.226**	0.000	−0.445**	0.000	−0.520**	0.000
FEV 1	−0.262**	0.000	−0.419**	0.000	−0.517**	0.000
FEV1%FVC	−0.171**	0.002	−0.100	0.245	−0.136	0.055
FEV1%VC MAX	−0.234**	0.000	−0.229**	0.007	−0.205**	0.004
PEF	−0.181**	0.001	−0.405**	0.000	−0.368**	0.000
FEF25	−0.216**	0.000	−0.374**	0.000	−0.378**	0.000
FEF50	−0.251**	0.000	−0.289**	0.001	−0.394**	0.000
FEF75	−0.315**	0.000	−0.372**	0.000	−0.386**	0.000
MMEF75/25	−0.292**	0.000	−0.333**	0.000	−0.434**	0.000
IC	−0.165**	0.004	−0.319**	0.000	−0.279**	0.000

(Continued)

Table 3. (Continued)

	CYSC					
	Total		Male		Female	
	r_p	*p*	r_p	*p*	r_p	*p*
MV	0.208**	0.000	0.124	0.159	0.193**	0.009
FEV3	−0.289**	0.000	−0.475**	0.000	−0.522**	0.000
FEV3%FVC	−0.220**	0.010	−0.096	0.431	−0.314**	0.009
VT	0.106	0.063	0.042	0.633	−0.037	0.624
BF	0.195**	0.001	0.169	0.069	0.294**	0.000
ERV	0.148**	0.010	−0.140	0.117	−0.332**	0.000
VC IN	−0.152**	0.005	−0.341**	0.000	−0.317**	0.000

ALB, albuminous; BF, breath frequency; BMI, body mass index; CTSB, cathepsin B; CYSC, cystatin C; DBP, diastolic blood pressure; ERV, expiratory reserve volume; FBG, fasting blood glucose; FEF25, forced expiratory flow at 25% of FVC; FEF50, forced expiratory flow at 50% of FVC; FEF75, forced expiratory flow at 75% of FVC; FEV 1, forced expiratory volume in 1 s; FEV1%FVC, the ratio of FEV1 and FVC; FEV1%VC, the ratio of FEV1 and vital capacity; FEV3%FVC, forced expiratory volume in 3 s/FVC; FEV3, Forced expiratory volume in three seconds; FVC, forced vital capacity; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IC, inspiratory capacity; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MMEF75/25, maximal mid-expiratory flow; MV, minute ventilation; PEF, peak expiratory flow; RBC, red blood cells; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VC IN, inspiratory vital capacity; VC MAX, maximum vital capacity; VT, tidal volume; WBC, white blood cells.
p values are from analysis of variance.
*Significant difference ($p < 0.05$).
**Significant difference ($p < 0.01$).

Lung matrix homeostasis depends partly on the regulation of proteolytic activities.[27] Previous research has shown that both CTSB and CYSC may play crucial roles in myofibrogenesis and lung fibrosis.[28–30] Although their exact functions remain to be clarified, CTSB is expressed by idiopathic pulmonary fibrosis (IPF) fibroblasts and is secreted into the extracellular space by CCD-19Lu cells. CYSC controls extracellular CTSB because its gene silencing restored their proteolytic activities. CTSB silencing was shown to induce a reduction in α-SMA expression, supporting the concept that CTSB may be involved in transforming growth factor beta 1 (TGF-β1)-driven differentiation of primary IPF fibroblasts. CTSB silencing also had a significant effect on α-SMA protein and mRNA levels during CCD-19Lu differentiation, promoting the generation and secretion of ECM proteins that are hallmarks of fibroblast differentiation in fibrotic disorders.[21] However, previous studies on CTSB and CYSC are limited to animal- or cell-based experiments. Questions on whether human CTSB and CYSC participate in the pathophysiology of an age-related pulmonary subclinical state in healthy populations, and whether CTSB and CYSC may be used as biomarkers for age-related pulmonary dysfunction, remain to be answered. Our previous study confirmed that serum CTSB levels were correlated with an age-related cardiovascular-renal subclinical state in a healthy Chinese population. We proposed a novel hypothesis that serum CYSC levels were correlated with an age-related pulmonary subclinical state, and thus may provide new ways to delay the progress and improve the treatment of age-related pulmonary dysfunction.

Lung function is routinely measured using pulmonary function tests, which are important clinical tools for evaluating the physical and mechanical properties of the respiratory system. Parameters measured include airway resistance, elasticity of the respiratory system, and the contractile forces of respiratory muscles. With aging, changes are not only observed in lung parenchyma, but also in chest wall shape and muscle forces, all of which affect pulmonary function. The aging process alters the intrinsic structure of the lung as well as of the supportive extrapulmonary structures. These structural changes lead to unfavorable

Table 4. Relationship between serum CTSB levels and pulmonary parameters using a stepwise multiple regression model.

	Model 1		Model 2		Model 3		Model 4	
	Beta	***P***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***
Total								
VC MAX	0.179**	0.001	0.255**	0.000	0.247**	0.000	0.034	0.379
FVC	0.189**	0.000	0.267**	0.000	0.263**	0.000	0.046	0.219
FEV 1	0.147**	0.006	0.231**	0.000	0.224**	0.000	0.028	0.470
PEF	0.201**	0.000	0.255**	0.000	0.257**	0.000	0.031	0.040
FEF25	0.160**	0.003	0.219**	0.000	0.197**	0.000	0.032	0.485
FEF50	0.008	0.882	0.105*	0.024	0.008	0.869	−0.040	0.405
MMEF75/25	0.002	0.968	0.117**	0.009	0.023	0.631	−0.026	0.562
BF	0.048	0.363	0.021	0.696	−0.032	0.594	0.099	0.094
IC	0.141*	0.012	0.178**	0.001	0.103	0.052	−0.019	0.718
MV	0.168**	0.001	0.137*	0.011	0.121*	0.028	0.080	0.176
FEV3	0.213**	0.007	0.261**	0.000	0.221**	0.001	0.017	0.751
VC IN	0.118*	0.027	0.182**	0.000	0.141**	0.004	−0.046	0.288
Male								
VC MAX	−0.051	0.536	0.022	0.734	0.038	0.577		
FVC	−0.055	0.506	0.021	0.738	0.038	0.561		
FEV 1	−0.084	0.310	−0.004	0.946	0.012	0.852		
PEF	−0.019	0.824	0.031	0.684	0.038	0.623		
FEF25	−0.040	0.631	0.014	0.856	0.019	0.807		
FEF50	−0.076	0.336	0.004	0.956	0.020	0.793		
MMEF75/25	−0.106	0.179	−0.009	0.893	0.020	0.773		
BF	0.204*	0.012	0.230**	0.005	0.222**	0.007		
IC	−0.057	0.515	−0.019	0.824	−0.052	0.529		
MV	0.131	0.108	0.103	0.212	0.123	0.169		
FEV3	−0.047	0.675	−0.044	0.575	−0.023	0.761		
VC IN	−0.105	0.207	−0.041	0.559	−0.040	0.584		
Female								
VC MAX	−0.089	0.202	0.011	0.834	0.004	0.941		
FVC	−0.074	0.294	0.030	0.547	0.040	0.454		
FEV 1	−0.094	0.180	0.010	0.837	0.018	0.735		

(Continued)

Table 4. (Continued)

	Model 1		Model 2		Model 3		Model 4	
	Beta	***P***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***
PEF	−0.066	0.344	0.011	0.853	0.045	0.487		
FEF25	−0.059	0.398	0.014	0.815	0.064	0.334		
FEF50	−0.190	0.004	−0.073	0.228	−0.060	0.365		
MMEF75/25	−0.159*	0.017	−0.027	0.642	−0.013	0.837		
BF	0.066	0.346	0.019	0.788	−0.070	0.370		
IC	−0.016	0.827	0.011	0.874	−0.014	0.849		
MV	0.105	0.135	0.082	0.257	0.057	0.448		
FEV3	0.011	0.923	0.106	0.226	0.091	0.320		
VC IN	−0.140*	0.045	−0.065	0.284	−0.057	0.385		

ALB, albuminous; BF, breath frequency; BMI, body mass index; CTSB, cathepsin B; CYSC, cystatin C; DBP, diastolic blood pressure; ERV, expiratory reserve volume; FBG, fasting blood glucose; FEF25, forced expiratory flow at 25% of FVC; FEF50, forced expiratory flow at 50% of FVC; FEF75, forced expiratory flow at 75% of FVC; FEV 1, forced expiratory volume in 1 s; FEV1%FVC, the ratio of FEV1 and FVC; FEV1%VC, the ratio of FEV1 and vital capacity; FEV3%FVC, forced expiratory volume in 3 s/FVC; FEV3, Forced expiratory volume in three seconds; FVC, forced vital capacity; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IC, inspiratory capacity; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MMEF75/25, maximal mid-expiratory flow; MV, minute ventilation; PEF, peak expiratory flow; RBC, red blood cells; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VC IN, inspiratory vital capacity; VC MAX, maximum vital capacity; VT, tidal volume; WBC, white blood cells.
Beta, standardized coefficients. Model 1, unadjusted model; Model 2, adjusted for age; Model 3, fully adjusted except for gender; Model 4, fully adjusted for age, gender, weight, height, BMI, cigarette smoking, SBP, DBP, WBC, RBC, HGB, ALB, TG, TC, HDL, LDL, FBG, CYSC. Standardized coefficients and *p* values were outcomes from regression analyses.
*Significant difference ($p < 0.05$).
**Significant difference ($p < 0.01$).

respiratory mechanics associated with decreased expiratory flows, increased air trapping and closing volume, and decreased gas exchange.[27] Our findings confirmed previous studies that showed significant associations between age and pulmonary function. Participants were divided into two groups according to age. CTSB and CYSC levels increased significantly with age; however, pulmonary variables, except MV and BF, decreased with age ($p < 0.05$). CTSB and CYSC, through ECM remodeling and degrading of extracellular matrices, participate in lung parenchyma damage and fibrosis processes.[28–30] In this study, we found that serum CTSB was correlated only with BF in males after adjustment for possible confounders. After adjusting for all other possible confounders, CYSC remained significantly associated with IC, MV, FEV3%FVC, BF, ERV in all participants and exhibited significant and independent associations with FVC, IC, and FEV3 in males. CYSC is a sensitive indicator for several chronic inflammatory diseases. Previous research has confirmed that serum CYSC was correlated negatively with FEV1% predicted and FEV1/FVC in COPD patients.[27,28] In addition, CYSC was related to inspiratory muscle dysfunction in COPD, as maximal inspiratory pressures and sustained maximal inspiratory pressures were markedly reduced, with greater degrees of inflammation in COPD as expressed by higher levels of CYSC.[29] CYSC is a positive acute-phase reactant in COPD patients, and may indicate systemic inflammation during COPD progression. However, previous studies are limited to patients with diseases such as COPD. In our study, we analyzed correlations between serum CYSC concentrations and declines in normal, age-related lung functions in healthy people. We conclude that serum CYSC is independently correlated with normal age-related lung functions in healthy adults.

Table 5. Relationship between serum CYSC levels and pulmonary parameters using a stepwise multiple regression model.

	Model 1		Model 2		Model 3		Model 4	
	Beta	*P*	Beta	*p*	Beta	*p*	Beta	*p*
Total								
VC MAX	−0.237**	0.000	0.096	0.088	−0.015	0.699	−0.029	0.445
FVC	−0.243**	0.000	0.090	0.106	−0.012	0.749	−0.039	0.277
FEV 1	−0.286**	0.000	0.064	0.239	−0.008	0.847	−0.044	0.274
FEV1%FVC	−0.203**	0.000	−0.083	0.196	−0.084	0.211	−0.084	0.211
FEV1%VC MAX	−0.229**	0.000	−0.081	0.201	−0.068	0.295	−0.068	0.295
PEF	−0.211**	0.000	0.024	0.698	−0.085	0.104	−0.087	0.074
FEF25	−0.244**	0.000	0.013	0.832	−0.050	0.364	−0.091	0.080
FEF50	−0.255**	0.000	0.001	0.987	−0.035	0.545	−0.086	0.137
FEF75	−0.260**	0.000	0.024	0.676	0.000	0.999	−0.018	0.756
MMEF75/25	−0.285**	0.000	0.017	0.766	−0.018	0.737	−0.046	0.396
IC	−0.165**	0.004	−0.028	0.685	−0.240**	0.000	−0.240**	0.000
MV	0.171**	0.003	0.171**	0.003	0.174**	0.002	0.174**	0.002
FEV3	−0.318**	0.000	0.014	0.862	−0.011	0.855	−0.029	0.615
FEV3%FVC	−0.173*	0.042	−0.173*	0.042	−0.222*	0.013	−0.222*	0.013
VT	0.106	0.063	0.102	0.138	0.092	0.112	0.072	0.211
BF	0.159**	0.008	0.159**	0.008	0.225**	0.000	0.225**	0.000
ERV	−0.122*	0.036	0.150*	0.021	0.164**	0.008	0.156*	0.011
VC IN	−0.166**	0.002	0.127*	0.032	0.025	0.603	0.015	0.753
Male								
VC MAX	−0.376**	0.000	−0.050	0.503	−0.120	0.091		
FVC	−0.406**	0.000	−0.078	0.283	−0.145*	0.037		
FEV 1	−0.389**	0.000	−0.046	0.519	−0.084	0.232		
FEV1%FVC	−0.120	0.160	0.032	0.737	0.014	0.890		
FEV1%VC MAX	−0.179*	0.036	−0.012	0.901	0.033	0.727		
PEF	−0.384**	0.000	−0.188*	0.031	−0.151	0.093		
FEF25	−0.354**	0.000	−0.131	0.127	−0.099	0.269		
FEF50	−0.269**	0.001	−0.017	0.845	−0.018	0.841		
FEF75	−0.249**	0.003	0.049	0.553	0.012	0.890		
MMEF75/25	−0.302**	0.000	0.005	0.948	−0.016	0.846		
IC	−0.319**	0.000	−0.319**	0.000	−0.267**	0.001		
MV	0.106	0.229	0.039	0.703	0.090	0.318		

(Continued)

Table 5. (Continued)

	Model 1		Model 2		Model 3		Model 4	
	Beta	***P***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***
FEV3	−0.495**	0.000	−0.203*	0.018	−0.219**	0.008		
FEV3%FVC	−0.129	0.289	−0.168	0.195	−0.251	0.102		
VT	0.031	0.723	−0.036	0.725	0.011	0.937		
BF	0.130	0.163	0.134	0.201	0.063	0.512		
ERV	−0.121	0.175	0.150	0.106	0.155	0.088		
VC IN	−0.306**	0.000	−0.014	0.861	−0.055	0.488		
Female								
VC MAX	−0.448**	0.000	−0.020	0.742	−0.008	0.882		
FVC	−0.453**	0.000	−0.017	0.781	0.011	0.841		
FEV 1	−0.487**	0.000	−0.070	0.246	−0.065	0.265		
FEV1%FVC	−0.220**	0.002	−0.126	0.146	−0.136	0.120		
FEV1%VC MAX	−0.246**	0.000	−0.116	0.172	−0.127	0.147		
PEF	−0.384**	0.000	−0.051	0.484	−0.045	0.530		
FEF25	−0.386**	0.000	−0.071	0.333	−0.086	0.240		
FEF50	−0.391**	0.000	−0.139	0.070	−0.139	0.079		
FEF75	−0.352**	0.000	−0.100	0.201	−0.109	0.174		
MMEF75/25	−0.410**	0.000	−0.122	0.100	−0.126	0.099		
IC	−0.279**	0.000	−0.055	0.525	−0.093	0.225		
MV	0.178*	0.017	0.178*	0.017	0.118	0.134		
FEV3	−0.421**	0.000	0.075	0.449	0.049	0.612		
FEV3%FVC	−0.222	0.069	−0.256	0.122	−0.052	0.706		
VT	0.078	0.299	0.150	0.104	0.073	0.342		
BF	0.260**	0.001	0.260**	0.001	0.047	0.612		
ERV	−0.280**	0.000	0.001	0.988	0.067	0.418		
VC IN	−0.318**	0.000	0.033	0.650	0.002	0.974		

ALB, albuminous; BF, breath frequency; BMI, body mass index; CTSB, cathepsin B; CYSC, cystatin C; DBP, diastolic blood pressure; ERV, expiratory reserve volume; FBG, fasting blood glucose; FEF25, forced expiratory flow at 25% of FVC; FEF50, forced expiratory flow at 50% of FVC; FEF75, forced expiratory flow at 75% of FVC; FEV 1, forced expiratory volume in 1 s; FEV1%FVC, the ratio of FEV1 and FVC; FEV1%VC, the ratio of FEV1 and vital capacity; FEV3%FVC, forced expiratory volume in 3 s/FVC; FEV3, Forced expiratory volume in three seconds; FVC, forced vital capacity; HDL-C, high-density lipoprotein cholesterol; HGB, hemoglobin; IC, inspiratory capacity; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; MMEF75/25, maximal mid-expiratory flow; MV, minute ventilation; PEF, peak expiratory flow; RBC, red blood cells; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; VC IN, inspiratory vital capacity; VC MAX, maximum vital capacity; VT, tidal volume; WBC, white blood cells.
Beta, standardized coefficients. Model 1, unadjusted model; Model 2, adjusted for age; Model 3, fully adjusted except for gender; Model 4, fully adjusted for age, gender, weight, height, BMI, cigarette smoking, SBP, DBP, WBC, RBC, HGB, ALB, TG, TC, HDL, LDL, FBG, CYSC. Standardized coefficients and *p* values were outcomes from regression analyses.
*Significant difference ($p < 0.05$).
**Significant difference ($p < 0.01$).

However, there were several limitations to this study. First, this research was a cross-sectional study, with participants recruited from communities in northern Chinese cities; therefore, the general implications of the data are limited. A longitudinally designed study, with participants from different geographic areas in China, is required to confirm these findings. Second, the number of participants was relatively low, especially in the elderly age group. Also, there were more females (55.6%) when compared with males (44.3%). Third, this research was limited in terms of the choice of study measurements and variables. In addition, some variables were not analyzed because of measurement limitation. Thus, further studies should be performed to confirm and improve these findings.

In summary, this is the first study to examine associations between serum CTSB and CYSC levels with declines in normal age-related lung functions. Our results confirm an association between serum CYSC and an age-related pulmonary subclinical state. However, the association between serum CTSB and lung function was not well confirmed. Age-related increases in CYSC levels were independently correlated with declines in normal age-related lung functions. Measurement of CYSC serum, or in combination with other metabolic and endocrine biomarkers, may provide evidence for predicting pulmonary functions in healthy people, especially in elderly adults.

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Author contribution(s)

Nan Wang: Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Resources; Software; Writing-original draft; Writing-review & editing.

Yajun Yuan: Data curation; Methodology; Software; Validation; Visualization; Writing-review & editing.

Xiaojuan Bai: Formal analysis; Funding acquisition; Investigation; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing.

Wen Han: Investigation; Methodology; Writing-original draft.

Lulu Han: Conceptualization; Data curation; Methodology; Project administration; Resources; Writing-original draft.

Bijuan Qing: Methodology; Writing-review & editing.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

The reviews of this paper are available via the supplemental material section.

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