Determination of Clinical Cut-Off Values for Serum Cystatin C Levels to Predict Ischemic Stroke Risk

Youyi Wang, BA,* Ying Zhang, BA,* Qinghua Ma, MPH,† Congju Wang, MPH,‡ Yong Xu, MPH,* Hongpeng Sun, PhD,* and Yana Ma, MPH, PhD*

Background: The association between cystatin C and risk of ischemic stroke is inconsistent and the cut-off values of cystatin C are diverse in different articles. We aimed to investigate the association between cystatin C levels and the development of ischemic stroke and to explore the clinical cut-off values of serum cystatin C levels for ischemic stroke. *Methods*: This prospective cohort study included 7658 participants from the China Health and Retirement Longitudinal Study who were free of cardiovascular diseases and cancer at baseline. A decision-tree model was used to find reasonable cut-off values for cystatin C levels. Logistic regression models were used to analyze the association between different levels of cystatin C and the risk of ischemic stroke. *Results*: The whole cohort was divided into the following 3 groups according to the decision tree: group-low (<.901 mg/L), group-moderate (.901~1.235 mg/L), and group-high (>1.235 mg/L). After 4 years of follow-up, we identified 156 cases of ischemic stroke. After adjusting for potential confounding factors, the odds ratios (95% confidence intervals) of ischemic stroke were 1.637 (1.048-2.556) for group-moderate and 2.326 (1.285-4.210) for group-high) compared with the low group of cystatin C. Subgroup analyses showed that the association between cystatin C levels and the incidence of ischemic stroke was more pronounced in males or old people than in females or young people. *Conclusions*: We found 2 suitable cut-off values for serum cystatin C levels and found that high levels of cystatin C were associated with an increased risk of ischemic stroke.

Key Words: Cystatin C levels—risk—ischemic stroke—cohort study—decision-tree model

Introduction

Cerebral stroke leads to a group of acute cerebral circulatory disorders caused by limited or comprehensive brain functional impairment syndromes[1] and includes ischemic and hemorrhagic strokes.[2,3] Data from the *Chinese Stroke Prevention and Control Report (2015)* show that 15% of the Chinese population over 40 years old are at high risk of cerebral stroke. Among the different types of stroke, ischemic stroke constituted 77.8% of all strokes, and intracerebral hemorrhage constituted 15.8% of all strokes.[3] Cerebral stroke has the characteristics of high morbidity, mortality, and disability.[4] Once a cerebral stroke occurs, few people can fully recover. Because of the

From the *Department of Child Health, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, School of Public Health, Soochow University, Suzhou, PR China; †The 3rd People's Hospital of Xiangcheng District, Suzhou, PR China; and ‡Centers for Disease Control and Prevention of Suzhou High-tech Zone, Beijing, PR China.

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Address correspondence to Hongpeng Sun, PhD and Yana Ma, MPH, PhD, Department of Child Health, Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, School of Public Health, Soochow University, 199 Ren-ai Rd, SuZhou City, Jiangsu Province 215123, China. E-mails: hpsun@suda.edu.cn, yanama@suda.edu.cn.

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lack of effective treatments, prevention is now considered the best course of action.[5] It is urgent to screen and intervene in cerebral stroke risk as soon as possible.

Recent studies have shown that cerebrovascular diseases may be associated with impaired renal function. Renal injury is a common complication in patients with acute stroke.[6] There is growing evidence that chronic kidney disease is also a risk factor for stroke. Cerebral nephropathy and chronic nephropathy may have the same risk factors and pathophysiological mechanisms.[7] Because kidney and cerebrovascular diseases have similar anatomical and functional characteristics and are uniquely susceptible to vascular damage, kidney damage can predict the existence and severity of small-vessel disease.[8] Cystatin C is a cysteine proteinase inhibitor that can be used as an endogenous marker to reflect glomerular filtration function.[9] Research data have shown that the cystatin C level has a higher sensitivity and specificity than the glomerular filtration rate (GFR),[10] serum blood urea nitrogen level, and creatinine (Cr) level since it is of great value in the evaluation of GFR. It is significantly negatively correlated with GFR, and when GFR is slightly impaired, serum cystatin C level is significantly increased and gradually increased as the disease worsened.[11] Therefore cystatin C concentration has been postulated to be an improved marker of GFR compared with the serum Cr level. A case-control study of the relationship between plasma cysteine protease inhibitor C levels and intracerebral hemorrhage has shown that elevated plasma cysteine protease inhibitor C levels are a risk factor for intracerebral hemorrhage.[12]

Many studies have shown that the early functional outcome of ischemic stroke patients is related to the level of cystatin C. Cystatin C is an important determinant of endogenous neuroprotection and may be an important predictor of the functional prognosis of ischemic stroke patients.[13] It has also been suggested that elevated cystatin C levels are independently associated with ischemic stroke and are strong predictors of cardiovascular events and mortality risk.[14] However, some teams do not support the causal role of cystatin C in the etiology of ischemic stroke.[15] Therefore, the purpose of our study was to explore the relationship between the level of cystatin C and the incidence of ischemic stroke in a prospective study. Moreover, we want to find appropriate clinical cutoff values that can effectively predict the risk of ischemic stroke in the population and thus serve as an auxiliary means for diagnosing ischemic stroke.

Methods

Study Population

This study was conducted on the basis of the CHARLS, which is a nationally representative longitudinal survey conducted by the Institute of Social Science Survey, Peking University. The CHARLS examines the health changes and influencing factors of middle-aged and elderly populations in China. In this study, follow-up data have been collected through face-to-face computer-assisted personal interviews by 2 surveys in 2013-2014 and 2015-2016. This survey was divided into 8 parts, and our study focused on the part concerning health status and functioning. The inclusion criteria of this study were adults over 45 years old who were willing to participate in this survey and could be collected serum samples. All investigations were conducted with the informed consent of the participants.

The number of people investigated in 2011 were 11,635. In the first step, we included 8169 people with cystatin C monitoring as a baseline. The missing data and the absence of cystatin C monitoring data were excluded. Second, we excluded participants with a history of ischemic stroke, cancer, myocardial infarction, peripheral arterial disease, heart failure, or abdominal aortic aneurysm, and other cardiovascular diseases before baseline because a prior diagnosis of or active treatment for cancer and cardiovascular disease could influence the occurrence of ischemic stroke. Thus 7986 participants remained in the current analysis. As we used retrospective cohort studies, participants who met the inclusion criteria must have completed the 2013-2014 or 2015-2016 survey. In this study, 328 participants did not complete the follow-up. We also searched for the cause of death in the lost participants. If the participants died of an ischemic stroke, we included these people in the study. If they did not complete the follow-up for unknown reasons, these people were excluded. In the end, a total of 7658 participants were included in this analysis. The detailed screening process was shown in the Figure 1.

Diagnosis of Ischemic Stroke

The outcome was the first occurrence of ischemic stroke according to tenth versions of International Classification of Diseases (I63), which identified by clinical symptoms and imaging examination that includes head computed tomography, magnetic resonance imaging scan, and digital subtraction angiography.[16,17]

Blood Samples Collection and Measurement of Serum Cystatin C Level

All the investigators performed the testing in a double-blinded manner for the case of serum cystatin C measurements. There are unified norms and requirements for the treatment of serum samples. In this project, a total of 8 mL of anticoagulated blood samples were collected intravenously from each subject, of which 4 mL tubes were used to collect plasma and white blood cells, and 2 mL tubes were used to determine the HbA1c and blood routine. All blood samples were processed at township health centers. For the 4 mL of blood used to separate plasma and leukocytes, the blood sample was centrifuged within 2 hours

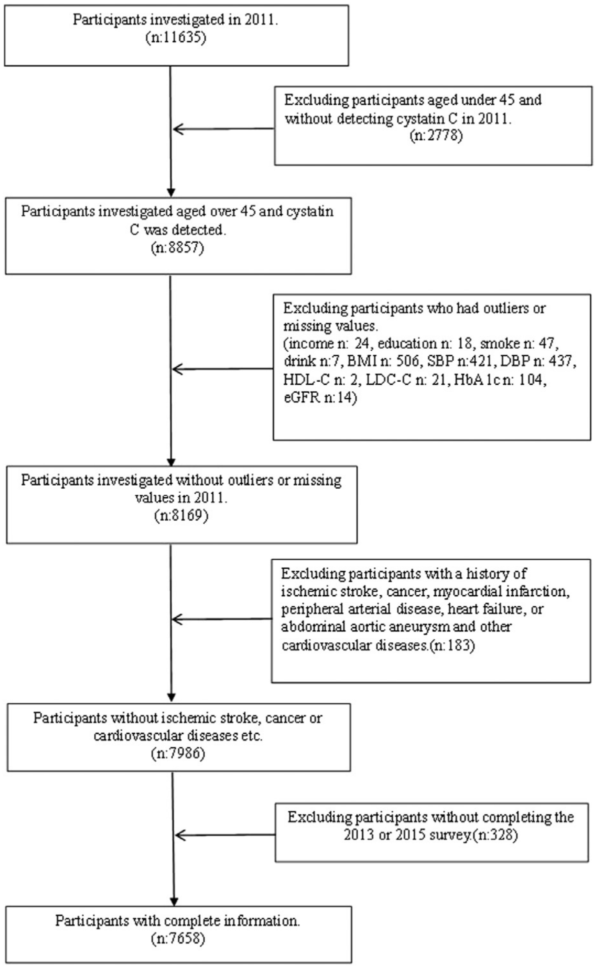

Figure 1. *Screening flow.*

after collection. After the venous blood was separated into plasma and the buffy coat, the plasma was then stored in three .5 mL cryovials, and the buffy coat was stored in a separate cryovial. These cryovials were then immediately frozen and stored at −20°C and were transported to the Chinese Center For Disease Control And Prevention laboratories in Beijing within 2 weeks. The laboratory detected cystatin C with particle enhanced turbidimetric assay.[18]

Other Measurements

The assessment of renal function was based on the estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration Cr equation with an adjusted coefficient of 1.1 for the Chinese population.[19]

Statistical Analysis

To find reasonable cut-off values for cystatin C levels, a decision-tree model was used. The original data set was randomly split into a training data set and a validation data set. In total, 70% of the original data was used as training data to find the best cut-off values for serum cystatin C. A chi-squared automatic interaction detection was used to develop the decision-tree model. Its results were illustrated using a tree structure. The root was at the top, and the leaves indicating an outcome category are put at the bottom. At the root, all classifications were mixed, representing the original data set. We set the depth of the decision tree no more than 2, so it will only include 2 factors for the analysis. Confusion matrix and ROC curve were used as the standards of model evaluation. The other 30% was used as validation data to directly judge the effect of cut-off values, in order to optimize the model with better effect.

The continuous variables were expressed as the mean ± standard deviation, and ANOVA was used to test for significant differences between groups. Categorical variables (such as sex and current smoking status) were expressed as constituent ratios, and chi-square tests were used to determine significant differences between groups.[20] Then, we performed a univariate logistic model to determine whether there is an association between the cystatin C level and ischemic stroke. A multivariate logistic regression analysis was performed to control for confounding factors. Considering the collinearity between variables, we included age, gender, and cystatin C in model 1. In the same way, we included age, sex, income, education, smoke, drink, body mass index, systolic blood pressure, blood glucose, total cholesterol (TC), high-sensitivity C-reactive protein, glycosylated hemoglobin (HbA1c), eGFR and cystatin C in model 2. We also performed sensitivity analyses and subgroup analyses of cystatin C levels. The results of the logistic regression models were presented using odds ratios (ORs) and 95% confidence intervals (95% CIs). All statistical analyses were conducted with the statistical software package SAS version 9.4. The threshold level for statistical significance was established at P less than .05.

Results

Cut-Off Values according to the Decision-Tree Model

The cohort included 7658 participants who had complete follow-up information. After a follow-up of 4 years, we identified that 156 (7 people of them died of ischemic stroke) of the patients eventually had an ischemic stroke. All the participants were divided randomly into a training data set (n = 5361) and a validation data set (n = 2297). Based on the decision-tree model, 2 clinical cut-off values for cystatin C levels were identified in the training data set. When the concentration of cystatin C was lower than .901 mg/L, 34.7% (n = 2654) of participants belonged to low-risk group for ischemic stroke (group-low). When the concentration of cystatin C was between .901 and 1.235 mg/L, 51.4% (n = 3937) of participants belonged to moderate-risk group for ischemic stroke (group-moderate). When the concentration of cystatin C was higher than 1.235 mg/L, 13.9% (n = 1067) of

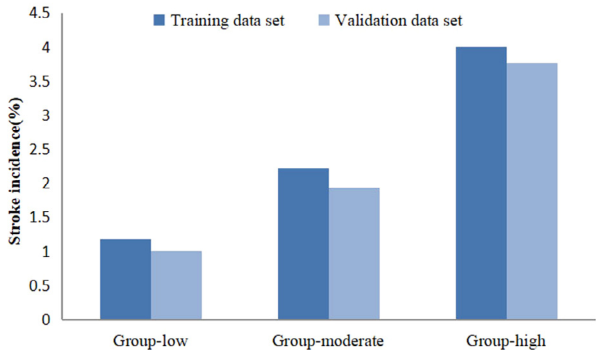

Figure 2. *Stroke incidence between 2011 and 2015.*

participants belonged to high-risk group for ischemic stroke (group-high). The incidence of ischemic stroke for the training data set was 1.18% in group-low, 2.22% in group-moderate, and 4.01% in group-high, that of the validation data set was 1.01%, 1.94%, and 3.77%, respectively (Fig 2). Table 1 summarized the baseline features of the whole cohort, in which patients with high concentrations of cystatin C tended to be old, male, and current smokers and had a low baseline eGFR ($P < .0001$).

Multivariate Logistic Regression Analysis between the Risk of Ischemic Stroke and the Cystatin C Level

As shown in Table 2, high levels of cystatin C were associated with the incidence of ischemic stroke in model 1. The OR (95% CIs) between group-low and group-moderate was 1.907 (1.253-2.902), and the OR (95% CIs) between group-low and group-high was 3.584 (2.231-5.758). After adjusting for confounding factors, the OR (95% CIs) between group-low and group-moderate was 1.637 (1.048-2.556), and the OR (95% CIs) between group-low and group-high was 2.326 (1.285-4.210). The level of cystatin C had a linear trend with the risk of ischemic stroke (P for trend = .0049).

Sensitivity Analysis and Subgroup Analysis

By excluding participants who had hypertension, group-high had a relationship with the incidence of ischemic stroke. The OR between group-low and group-high was 2.861 (1.258-6.507). Similar results were also observed by excluding patients who had diabetes and kidney disease, as shown in Table 3. In subgroup analyses stratified by sex, the association between the level of cystatin C and the incidence of ischemic stroke was more pronounced in the male population than in the female population (Table 4). After stratification by age, we found that the risk of ischemic stroke was higher in the upper middle age group than in the lower age group.

Discussion

In this prospective study of 7658 participants without a history of ischemic stroke, we determined 2 cut-off values for the level of cystatin C. The low truncation value of for the cystatin C level was .901 mg/L, and the high was 1.235 mg/L. Compared with a low level of cystatin C, people with a high level had an increased risk of ischemic

Table 1. *Baseline characteristics among 7658 Chinese participants according to cut-off values in 2011*

	Group-low	Group-moderate	Group-high	P value
N	2654 (34.7)	3937 (51.4)	1067 (13.9)	
Age, years	55.8 ± .2	60.8 ± .1	69.2 ± .3	<.0001
Female	1731 (65.9)	1877 (48.2)	420 (39.7)	<.0001
Income, dollar	3995 ± 165.7	3462 ± 124.8	2999 ± 138.4	.0007
Education (not completed high-school)	1741 (66.3)	2778 (71.3)	856 (80.9)	<.0001
Current smoker	561 (21.4)	1326 (34.0)	415 (39.2)	<.0001
Current drinker	944 (35.9)	1711 (43.9)	460 (43.5)	<.0001
BMI, kg/m^2	24.0 ± .1	23.3 ± .1	22.7 ± .1	<.0001
SBP, mmHg	131.0 ± .9	134.1 ± .8	143.2 ± 2.0	<.0001
DBP, mmHg	75.9 ± .2	75.9 ± .2	75.9 ± .4	.9970
GLU, mg/dL	113.7 ± .9	107.4 ± .4	110.1 ± 1.1	<.0001
TC, mg/dL	197.5 ± .8	191.9 ± .6	189.1 ± 1.2	<.0001
TG, mg/dL	146.4 ± 2.1	125.1 ± 1.4	115.7 ± 2.1	<.0001
HDL-C, mg/dL	50.1 ± .3	52.0 ± .2	52.0 ± .5	<.0001
LDL-C, mg/dL	117.3 ± .7	116.5 ± .6	115.2 ± 1.1	.2436
hsCRP, mg/L	2.0 ± .1	2.6 ± .1	4.3 ± .3	<.0001
HbA1c, %	5.3 ± 0.0	5.2 ± 0.0	5.3 ± 0.0	<.0001
eGFR, mL/min/1.73m^2	101.1 ± .2	94.7 ± .1	83.5 ± .3	<.0001

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; GLU, blood glucose; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Data are presented as number (percentage) or mean ± standard deviation.

Table 2. *Multivariate logistic regression for 3 groups of participants*

	Multivariate logistic regression analysis		
	Unadjusted	Model 1	Model 2
Group (low)	1 (reference)	1	1
Group (low-moderate)	1.907 (1.253-2.902)	1.661 (1.077-2.562)	1.637 (1.048-2.556)
Group (low-high)	3.584 (2.231-5.758)	2.619 (1.531-4.480)	2.326 (1.285-4.210)
P for trend	<.0001	.0004	.0049

Data are presented as odds ratio (95% confidence interval).

Grouping scope of cystatin C according to decision tree model: Group-low: <.901mg/L; Group-moderate: .901mg/L-1.235mg/L; Group-high: >1.235mg/L.

P value is for test of linear trend.

Model 1: adjusted for age, sex.

Model 2: adjusted for age, sex, income, education, smoke, drink, BMI, SBP, GLU, TC, hsCRP, HbA1c, and eGFR.

stroke. This association was independent of several potential confounding factors, including age, sex, smoking, drinking, body mass index, glucose level, total cholesterol, high-sensitivity C-reactive protein level, glycated hemoglobin level, income, education, systolic blood pressure, and eGFR. In addition, males or old people with high levels of cystatin C were more likely to have an ischemic stroke than females or young people.

In our study, the cut-off values of cystatin C levels were analyzed by a decision tree-model, which is a different method from those of other studies. For example, in Huang GX's research, the study population was divided into 4 groups based on the following quartile analysis of cystatin C levels, where the concentrations were: low (<.75 mg/L), intermediate (.75-1 mg/L), high (1-1.25 mg/L), and very high (>1.25 mg/L). Compared with the control group, the cystatin C levels were significantly increased in patients with acute ischemic stroke (AIS).[14] Additionally, Yang B divided the population into 5 groups based on serum cystatin C levels; compared with the first quintile, the ORs of risk for AIS in the fourth quintile and fifth quintile were 1.92 (1.08-3.40) and 2.88 (1.49-5.54), respectively.[21] To judge whether the method of dividing cut-off values according to the decision-tree model was reliable, we created another set of cut-off values based on tertiles. The low cut-off value of the cystatin C level was .900 mg/L, and the high cut-off value was 1.070 mg/L. According to a goodness-of-fit model (AIC,

Table 3. *OR (95% CIs) for risk of stroke (sensitivity analysis)*

		Model 1		Model 2	
	N	Group (low-moderate)	Group (low-high)	Group (low-moderate)	Group (low-high)
Excluding participants who had hypertension	5664	1.406 (.753-2.624)	2.940 (1.390-6.219)	1.410 (.742-2.679)	2.861 (1.258-6.507)
Excluding participants who had diabetes	7221	1.619 (1.025-2.559)	2.925 (1.676-5.103)	1.562 (.978-2.495)	2.516 (1.362-4.647)
Excluding participants who had kidney disease	7090	1.557 (.997-2.433)	2.502 (1.427-4.387)	1.538 (.970-2.438)	2.166 (1.164-4.031)

Table 4. *OR (95% CIs) for risk of stroke (subgroup analysis)*

		Model 1		Model 2	
	N	Group (low-moderate)	Group (low-high)	Group (low-moderate)	Group (low-high)
Women	4070	1.504 (.857-2.640)	1.878 (.816-4.323)	1.394 (.777-2.501)	1.545 (.622-3.837)
Men	3588	2.136 (1.035-4.407)	3.642 (1.628-8.150)	2.239 (1.065-4.708)	3.702 (1.528-8.971)
$45 \leq$ age < 55	2409	2.125 (.979-4.610)	1.670 (.208-13.419)	2.203 (.972-4.996)	1.696 (.191-15.027)
$55 \leq$ age <65	2813	1.324 (.694-2.529)	3.716 (1.680-8.219)	1.271 (.649-2.489)	2.946 (1.168-7.430)
Age ≥ 65	2436	1.926 (.749-4.953)	2.552 (.974-6.691)	1.856 (.712-4.841)	2.201 (.771-6.281)

Table 5. *Comparison of goodness-of-fit between decision tree model and tertile method*

	Unadjusted		Model 1		Model 2		
	Decision tree	Tertile	Decision tree	Tertile	Decision tree	Tertile	Model 2_1 (excluded cystatin C)
AUC	.611	.612	.634	.636	.659	.657	.647
AIC	1501.227	1503.321	1498.816	1500.340	1507.637	1508.501	1511.81
SC	1522.058	1524.152	1533.534	1535.058	1618.734	1619.597	1609.019
−2Log L	1495.227	1497.321	1488.816	1490.340	1475.637	1476.501	1483.81

SC, -2Log L), the cut-off values determined by the decision-tree model were better. Table 5 showed the specific data. We found that the cut-off values obtained by the above segmentation methods were variable and unstable. The cut-off values obtained by the decision-tree algorithm had certain extrapolation properties and can be applied to clinical diagnosis.

Our results are consistent with those of previous studies. In the large-scale study from Shlipak et al, they showed that the high-risk group (over 1.29 mg/L of cystatin C) had an approximately 50% higher risk for stroke than the low-risk group (≤.99 mg/L).[22] Our results are also supported by a previous study that showed that an elevated level of cystatin C was an independent factor that affected the occurrence of ischemic stroke.[23] Some studies, however, have shown different results. For stroke patients, cystatin C could be an endogenous neuroprotective factor, and it was also applied as a novel treatment for stroke since it maintains the integrity of the lysosomal membrane integrity.[13] There was also a Mendelian randomized analysis that did not support a causal role for cystatin C in the etiology of cardiovascular disease. But our prospective study provided new evidence for cystatin C as a risk factor for ischemic stroke.

When using the decision-tree model, we divided the population into 3 groups only according to cystatin C level. After exploring the interaction and adjusting for the confounding factors, we found that high levels of cystatin C were still an independent risk factor for ischemic stroke. It suggested that when the serum cystatin C concentration was greater than .901 mg/L, the risk of ischemic stroke increased significantly. This finding will help clinicians to predict ischemic stroke in advance and guide people to take preventive measures. Meanwhile, measuring the serum cystatin C concentration is more convenient and cheaper than performing the other methods, which reduces the operating costs for medical institutions. To discuss whether there was a difference in cut-off values for serum cystatin C levels between the male and female population, we used the same decision tree, and found the cut-off values for cystatin C in groups of male and female, respectively. The low cut-off value of the cystatin C level in male was .900 mg/L, and the high cut-off value was 1.235 mg/L, while in female was .957 mg/L and 1.458 mg/L. The results showed that the cut-off values for cystatin C in males was similar to that in the general population, and the cut-off values in females was higher than that in males. To be on the safe side and give early warning of risk of stroke, we chose the lower values as the clinical cut-off values for cystatin C.

The mechanism of ischemic stroke may be explained by the following reasons. Firstly, Cerebral arterial stenosis is one of the main causes of cerebral stroke.[24] Studies have shown that elevated serum cystatin C levels in patients with AIS are significantly and positively correlated with cerebral arterial stenosis. Secondly, cystatin C plays an important role in the pathogenesis of atherosclerosis. Some studies support the idea that cystatin C may directly induce and promote vascular smooth muscle cell proliferation and apoptosis, leukocyte adhesion and proliferation, cytokine release, macrophage chemotaxis, low-density lipoprotein oxidation, and lipid deposition in the arterial wall.[25] Thirdly, a study showed that high cystatin C levels are associated with a high concentration of CRP, which is a classic indicator of inflammation.[26]

Nowadays there is plenty of evidence that estrogen is protective in many tissues, including the brain, fatty tissue, heart, and vascular systems.[27,28] In the context of ischemic stroke, estrogen has a high neuroprotective effect on multiple levels, including inhibiting the pathology of stroke risk factors and regulating adiposity through the antiatherogenic effect of the vascular system. Estrogen also improves stroke pathology through direct neuroprotection of brain and glial cells during coronary vasodilation and ischemia.[29] So that's why females are more likely to have ischemic stroke than males, which will expect to be confirmed in further study. Age is an important risk factor for stroke among a series of demographic factors.[30] In previous studies on stroke patterns in China, researchers found that stroke incidence and mortality rates rose rapidly with age, which is consistent with the subgroup analysis of our paper. This phenomenon can be explained in the following 2 ways. Firstly, the elderly are at high risk of microvascular disease, so they're more likely to have kidney disease and stroke. Secondly, the incidence of stroke in the elderly group was high and the statistical power was high.

Several limitations of this study warrant acknowledgment. First, the information of the current study was derived from the CHARLS data. This nationally representative longitudinal survey for health information covers only a subset of the population. There were no external data used to verify our conclusions. Thus, the conclusions may not be generalizable to other populations. However, we had conducted internal verification, indicating that the results were consistent. Second, there was only a single time point at 2011 for measuring the cystatin C levels, while serial measurements over time could had allowed to better understand the actual association between the plasma levels of this biomarker and the risk of developing ischemic stroke, this could influence the association between baseline cystatin C levels and the risk of ischemic stroke. Third, the association between ischemic stroke severity and cystatin C could not be established because of lacking of the National Institute of Health stroke scale score for patients with ischemic stroke. Fourth, although the number of people we included in the early period was as high as 11,635, not all of the participants with high cystatin C levels had follow-up ischemic stroke investigations, which may have led to an underestimation of the association between cystatin C levels and ischemic stroke.

Conclusions

In conclusion, we determined 2 cut-off values for cystatin C levels and found that the cystatin C level was significantly associated with the incidence of ischemic stroke in individuals. Monitoring cystatin C levels provides an important approach for identifying high-risk groups for ischemic stroke and helps to prevent the incidence of ischemic stroke in this population. The results obtained from this prospective observational study of a large community-dwelling cohort have important public health value as they demonstrate that the cystatin C level is a predictor of ischemic stroke incidence. Further follow-ups are needed to further explore the association between cystatin C levels and ischemic stroke.

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Authors' Contributions

H.P.S. designed the experiments. Y.Y.W. and Y.Z. managed statistical analysis and interpretation of the results. Y.Y. W. and Y.Z. drafted the manuscript. H.P.S. critically revised the manuscript. All gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declared that there was no conflict of interest.

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