International Journal of General Medicine

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ORIGINAL RESEARCH

Serum Cystatin C and 90-Day Neurological Functional Prognosis in Acute Ischemic Stroke: Insights from a Prospective Cohort and Mendelian Randomization

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Introduction: The relationship between cystatin C levels and 90-day neurological functional prognosis in acute ischemic stroke (AIS) is not fully understood. This study investigated this association prospectively and employed Mendelian randomization (MR) analysis to assess potential causality.

Methods: A prospective cohort study enrolled 786 patients with AIS admitted to a tertiary Stroke Center between 2021 and 2023. Serum cystatin C levels were measured within 48 hours of admission. Associations with adverse 90-day neurological functional outcome (modified Rankin Scale score > 2) were assessed using univariate and multivariable logistic regression. Cox regression models evaluated all-cause mortality during long-term follow-up. Mediation analysis examined the role of inflammatory mediators (Monocyte-to-Lymphocyte Ratio [MLR], Neutrophil-to-Lymphocyte Ratio [NLR], Systemic Inflammatory Response Index [SIRI], Systemic Immune-Inflammation Index [SII]) in the cohort. Causality was further evaluated using a two-sample MR approach with genetic instruments to infer the effect of cystatin C on ischemic stroke risk.

Results: Elevated cystatin C levels were independently associated with higher odds of adverse 90-day functional outcome (adjusted odds ratio [OR] = 2.13, 95% CI: 1.34–3.38, p=0.001) and increased mortality risk (adjusted hazard ratio [HR] = 1.97, 95% CI: 1.09–3.54, p=0.024). Mediation analysis identified systemic inflammation markers as significant mediators, accounting for 16.1% to 20.2% of the total effect of cystatin C on adverse prognosis. MR analysis provided evidence supporting a causal relationship, indicating that genetically predicted higher cystatin C levels were associated with an increased risk of ischemic stroke (OR = 1.10, 95% CI: 1.02–1.18, p=0.011).

Conclusion: Elevated cystatin C levels demonstrated a significant association with worse 90-day functional outcome and higher mortality in these patients, partially mediated through systemic inflammation. MR findings suggested a potential causal role of cystatin C in ischemic stroke pathogenesis. Cystatin C may represent a valuable integrated biomarker for both prognosticating outcomes in AIS and predicting ischemic stroke risk.

Keywords: cystatin c, acute ischemic stroke, 90-day neurological functional prognosis, Mendelian randomization, systemic inflammation

Received: 18 July 2025
Accepted: 8 October 2025
Published: 15 October 2025

Introduction

Ischemic stroke is a leading global cause of death and long-term disability, imposing a tremendous socioeconomic burden.[1] This devastating neurological disorder results from acute cerebral ischemia, leading to neuronal damage and cell death.[2,3] Given the scale of this challenge, there is an urgent clinical need for improved biomarkers to enhance early risk prediction and facilitate accurate prognostication.[4]

Cystatin C, a natural inhibitor of cysteine proteases, has become an important biomarker for cardiovascular and renal diseases.[5,6] Critically, elevated cystatin C levels are independently associated with an increased risk of stroke and are a stronger predictor of long-term stroke mortality than traditional renal markers (creatinine or eGFR).[7,8] This association may stem not only from its link to underlying renal impairment (a known stroke risk factor), but also from its direct involvement in vascular pathology relevant to stroke, such as blood pressure modulation via the renin-angiotensin system, endothelial dysfunction, atherosclerosis and inflammation.[9–11] However, its specific role in the acute pathophysiology of stroke itself requires further investigation.[12]

Current evidence indicates that elevated serum cystatin C is significantly associated with stroke incidence and poor long-term outcomes. The CHARLS cohort revealed its predictive value for incident stroke (HR = 1.202).[13] The NHANES data revealed a critical threshold for mortality at 1.24 mg/L of serum cystatin C, indicating that each 0.1 mg/L increase is associated with a 6.73 to 10.60-fold increase in the risk of all-cause and cardiovascular mortality.[14] However, much of the evidence supporting this association comes from public databases, and there are only a few real-world studies evaluating its ability to predict early functional recovery. In particular, research on 90-day outcomes following acute ischemic stroke is limited.[15] Furthermore, the presence of confounding factors limits the ability to make causal inferences from observational associations. Further research is needed to clarify the role of cystatin C in early neurological functional recovery in patients with AIS and to explore the underlying mechanisms, thereby providing more reliable evidence for clinical decision-making.

Therefore, we conducted a prospective cohort study to assess whether early serum cystatin C levels independently predict 90-day neurological functional outcome and mortality in AIS patients. Additionally, we utilized mediational analysis to explore potential underlying mechanisms. Given that cystatin C is associated with acute ischemic stroke and may be connected to inflammation, which is a critical factor in brain injury after a stroke, we will examine the mediating role of inflammatory markers. To strengthen causal inferences regarding stroke susceptibility, we employed Mendelian Randomization (MR) analysis leveraging genetic variants associated with cystatin C levels. This study aims to comprehensively evaluate cystatin C as a biomarker for predicting ischemic stroke risk and assessing acute prognosis. Ultimately, we seek to leverage this knowledge to enhance the assessment and management of ischemic stroke, thereby improving patient survival and quality of life.

Materials and Methods

Study Design and Study Population

We conducted a prospective cohort study at a tertiary Stroke Center of the Affiliated Hospital of Nanjing University of Chinese Medicine, focusing on patients diagnosed with AIS between January 2021 and November 2023. To be included in the final analysis, participants had to meet several specific criteria. First, they had to be at least 18 years old. Second, they had to have been diagnosed with AIS according to the standards set by the Chinese Clinical Practice Guidelines for Acute Ischemic Stroke Management, with confirmation of the presence of a stroke lesion through brain magnetic resonance imaging. Third, they were required to have been admitted to the hospital within 48 hours of symptom onset. The initial registry included 2,369 patients aged over 18 years. After applying the inclusion criteria, patients were excluded for the following reasons: missing exposure data (n=1,477), loss to follow-up (n=7), incomplete laboratory indicators (n=13), severe infections (n=16), cancer or malignant lesions (n=63) and kidney dysfunction or failure (n=7). As a result, we established a final study population of 786 patients (Figure 1).

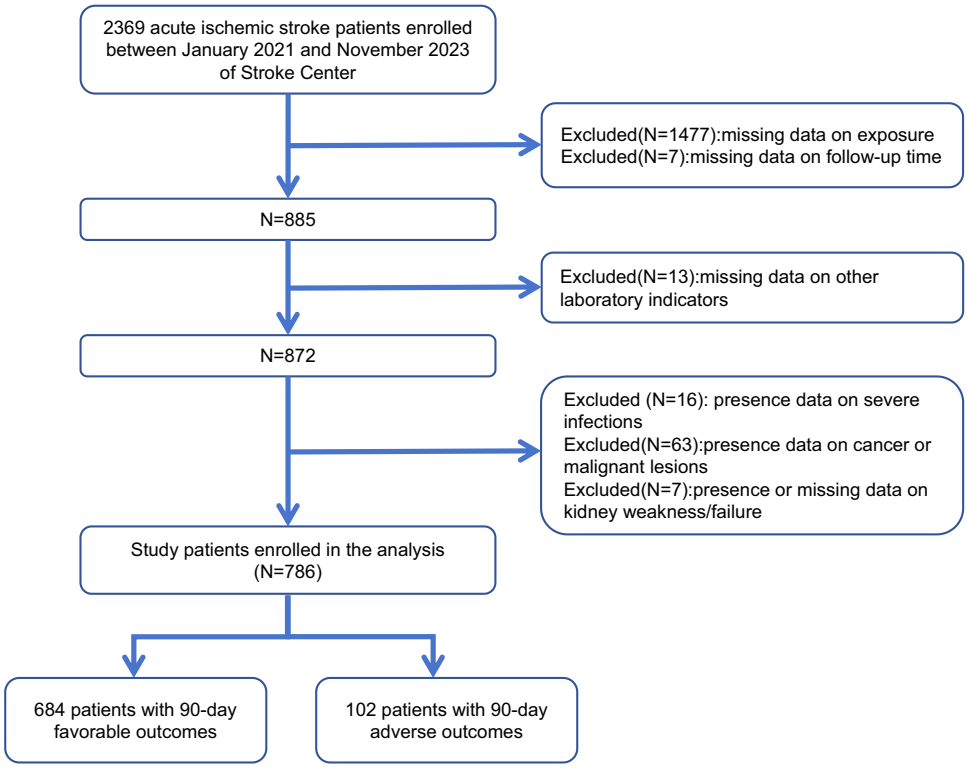

Figure 1 Flowchart of patients' selection in the Stroke Centre cohort.

Ethics Statement

The cohort study at the Stroke Center received approval from the Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine, in accordance with the ethical guidelines of the Helsinki Declaration (2017NL-012-01). Before enrollment, all participants in the cohort completed the informed consent process to ensure their voluntary participation.

Exposure

In the Study, serum cystatin C levels were the main exposure variable. Fasting blood samples were collected within 48 hours of each participant's hospital admission. All samples were analyzed in the central laboratory using standardized automated assays by certified technicians. While multiple measurements may have been obtained during the hospital stay, we selected the first sample for our analysis to maintain consistency.

Outcomes

The primary clinical outcome measure was the neurological functional status at 90 days, evaluated through the modified Rankin Scale (mRS).[16] The mRS scores were categorized into two groups: scores of 0–2 indicated favorable outcomes (functional independence), while scores of 3–6 represented adverse outcomes (including dependency or mortality).[17] A secondary outcome was all-cause mortality during the long-term follow-up period, which concluded in October 2024. Mortality data were collected through standardized hospital records and verified through national death registries when necessary. The causes of mortality included cardiovascular events, respiratory failure and other factors.

Covariates and Other Laboratory Indicators

The covariates included in the study were gender, age, hypertension, diabetes, coronary artery disease, smoking and alcohol use. Hypertension was defined as either a documented history of the condition, current use of antihypertensive medications, or having a systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg prior to

https://doi.org/10.2147/IJGM.S550462

admission. Diabetes mellitus was defined as a documented history of diabetes, current use of glucose-lowering medications, or an HbA1c level of ≥6.5% at admission. Coronary artery disease was defined as a documented history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, or angiographic evidence of coronary stenosis greater than 50%. Disease characteristics were assessed using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, modified Rankin Scale (mRS) scores, and National Institutes of Health Stroke Scale (NIHSS) measurements obtained at admission.[18] Laboratory indicators included leukocytes, lymphocytes, neutrophils, monocytes, platelets, total cholesterol, HDL cholesterol, urea, creatinine levels and glomerular filtration rate (eGFR). The eGFR was calculated using the 2021 CKD-EPI equation without race adjustment.[19] Age, admission NIHSS score, admission mRS score and laboratory data were treated as constant parameters, while other demographic and vascular risk factors were categorized as binary variables for comparative analysis.

Definition of Inflammation Indexes

This research investigated the possible mediating function of four indicators associated with inflammation. These indexes included Monocyte-to-Lymphocyte Ratio (MLR), Neutrophil-to-Lymphocyte Ratio (NLR), the Systemic Inflammatory Response Index (SIRI) and the Systemic Immune-Inflammation Index (SII). Using standard complete blood count parameters to calculate these inflammatory biomarkers, the mathematical formulas are as follows:

MLR = Monocyte count/Lymphocyte count;

NLR = Neutrophil count/Lymphocyte count;

SIRI = (Monocyte count × Neutrophil count)/Lymphocyte count;

SII = (Platelet count × Neutrophil count)/Lymphocyte count.[20]

Statistical Analyses

This study employed descriptive statistics to analyze the baseline characteristics of participants in the Stroke Center cohort at the Affiliated Hospital of Nanjing University of Chinese Medicine. Categorical variables were summarized using frequencies and percentages (%), while continuous variables were expressed as means with standard deviations (SD). Due to the skewed distributions of the NIHSS and mRS scores, these were reported as median (Q1-Q3). To compare outcomes at 90 days, between-group differences were assessed, with P-values provided.

The effect of cystatin C levels on stroke occurrence and its relationship with the 90-day prognosis in ischemic stroke cases were examined using various statistical tests. The methodologies included logistic regression, multiple fractional polynomials (MFP), analysis of variance (ANOVA) and chi-square tests.

We subsequently analyzed the relationship between cystatin C levels and outcomes using univariate and multivariate logistic regression analyses within the cohort. Our initial analysis used an unadjusted model, followed by three models that included necessary adjustments. And cystatin C was categorized into two equal parts as a continuous variable, using the median value of 1.15 mg/L as the cutoff point, to provide more detailed insights into its relationship with outcomes. Due to the limited number of death events, no further classification was conducted. All-cause mortality was reported and analyzed as a distinct binary outcome. We calculated the odds ratio (OR) and hazard ratio (HR), 95% confidence intervals (CIs) and p-values.

Building on these initial findings, we investigated potential mechanistic pathways through mediation analysis. This analysis focused on the relationship between cystatin C levels and 90-day adverse outcomes following ischemic stroke. Four inflammatory indexes—MLR, NLR, SIRI and SII—were the focus of this analysis as potential mediators. We evaluated the total effect, mediation effect, direct effect and proportion mediated for each inflammatory marker. The estimates, 95% CI and p-values were recorded.

The statistical analysis for these segments of the study was performed using EmpowerStats (PC version) 5.2 for Windows (www.empowerstats.com) and R 4.4.1 software.

Mendelian Randomization and Sensitivity Analysis

The MR analytical workflow is illustrated in Figure 2. This study employed MR to investigate the causal relationship between cystatin C levels and ischemic stroke. We identified single nucleotide polymorphisms (SNPs) as instrumental variables, based on data from Genome-Wide Association Study (GWAS).[21]

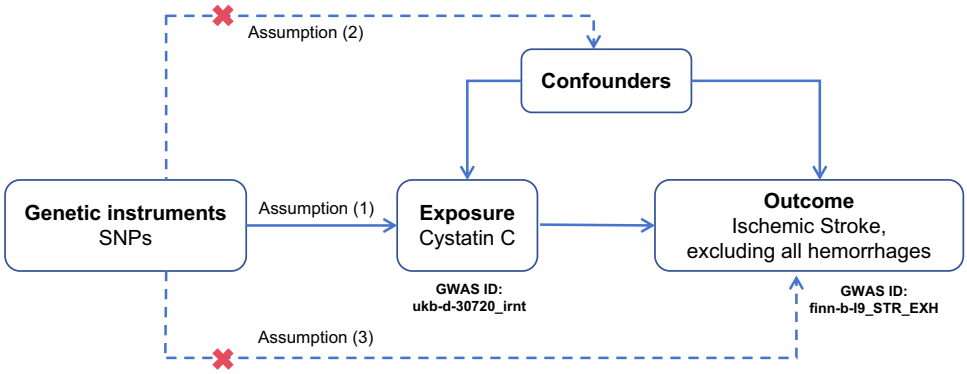

Figure 2 Flowchart of the MR analysis.
Notes: Assumption (1): There is a strong relationship between the genetic instruments and the exposure variable. Assumption (2): The genetic instruments are not correlated with any potential confounding variables. Assumption (3): The genetic instruments affect the outcome solely via their association with the exposure, without any direct causal links to the outcome.
Abbreviations: MR, Mendelian randomization; SNPs, Single Nucleotide Polymorphisms; GWAS, Genome-wide association study; IVW, Inverse Variance Weighted; PRESSO, Pleiotropy RESidual Sum and Outlier; P, P-value.

These SNPs were selected based on three criteria: relevance, independence and exclusivity.[22,23] The exposure data were obtained from the UK Biobank (Trait: Cystatin C - IEU OpenGWAS project) and the outcome data were sourced from the Finnish genotype and phenotype database (Finngen) (Trait: Ischaemic Stroke, excluding all haemorrhages - IEU OpenGWAS project). The retention of SNPs associated with cystatin C, which met stringent genome-wide significance criteria ($P < 5\times10^{-8}$), was subsequently subjected to linkage disequilibrium pruning to minimize genetic correlations. SNPs with F-statistics < 10 were excluded to maintain tool power. The exposure and outcome data were then incorporated into the databases. SNPs that were statistically significant contributors to ischemic stroke outcomes ($P < 5\times10^{-8}$) were excluded from the isolation restriction criterion.

Finally, we integrated information from both biobanks to made up a dataset containing 290 SNPs. Utilizing various techniques, including Inverse Variance Weighting (IVW), MR-Egger regression, weighted median estimation, heterogeneity assessment, MR-Egger intercept testing, MR-PRESSO analysis and leave-one-out analysis, we conducted a set of MR analyses and sensitivity analyses.

The statistical analysis for this segment of the study was performed using R version 4.4.1, along with specialized R packages, including "Two Sample MR" and "MR-PRESSO". We acknowledged that a P-value less than 0.05 is considered statistically significant.

Results

Baseline Characteristics of Participants

The cohort consisted of 786 patients diagnosed with AIS, of whom 684 had favorable outcomes (mRS score 0–2) and 102 had adverse outcomes (mRS score 3–6). And in the study, 11 patients were noted to have died during follow-up. Table 1 displays the fundamental characteristics of the participants involved in the Stroke Center cohort. Patients in the adverse outcome group were significantly older compared to those with favorable outcomes (75.0 ± 11.6 vs 67.1 ± 11.2 years, $P < 0.001$), had a higher proportion of males (52.9% vs 35.7%, $P < 0.001$), and exhibited elevated baseline cystatin C levels (1.4 ± 0.6 vs 1.2 ± 0.5 mg/L, $P < 0.001$). The adverse outcome group had a greater prevalence of vascular risk factors, including hypertension (87.3% vs 75.9%, $P = 0.010$) and diabetes (52.0% vs 39.5%, $P = 0.017$). Laboratory analyses indicated higher levels of inflammatory markers (white blood cell, neutrophil, and monocyte counts, all $P < 0.001$) and impaired renal function, as shown by lower eGFRcr (72.7 ± 23.0 vs 81.5 ± 20.0 mL/min/1.73m², $P < 0.001$) in these patients.

https://doi.org/10.2147/IJGM.S550462

Table 1 Baseline Characteristics of Patients from the Stroke Centre Cohort

	Total (N=786)	Outcomes		
		Favorable Outcomes (N=684)	Adverse Outcomes (N=102)	P Value
Exposure information				
Cystatin C, mean ± SD, mg/L	1.3 ± 0.5	1.2 ± 0.5	1.4 ± 0.6	<0.001
Demographic information				
Age, mean ± SD, years	68.1 ± 11.5	67.1 ± 11.2	75.0 ± 11.6	<0.001
Male, n (%)	298 (37.9%)	244 (35.7%)	54 (52.9%)	<0.001
Vascular risk factors				
Smoking, n (%)				<0.001
Yes	220 (28.0%)	206 (30.1%)	14 (13.7%)	
No	566 (72.0%)	478 (69.9%)	88 (86.3%)	
Alcohol, n (%)				0.022
Yes	150 (19.1%)	139 (20.3%)	11 (10.8%)	
No	636 (80.9%)	545 (79.7%)	91 (89.2%)	
Hypertension, n (%)				0.010
Yes	608 (77.4%)	519 (75.9%)	89 (87.3%)	
No	178 (22.6%)	165 (24.1%)	13 (12.7%)	
Diabetes, n (%)				0.017
Yes	323 (41.1%)	270 (39.5%)	53 (52.0%)	
No	463 (58.9%)	414 (60.5%)	49 (48.0%)	
Coronary Heart Disease, n (%)				0.002
Yes	62 (7.9%)	46 (6.7%)	16 (15.7%)	
No	724 (92.1%)	638 (93.3%)	86 (84.3%)	
Disease characteristics				
TOAST, n (%)				<0.001
LAA	382 (48.6%)	317 (46.3%)	65 (63.7%)	
CE	54 (6.9%)	42 (6.1%)	12 (11.8%)	
SAO	338 (43.0%)	315 (46.1%)	23 (22.5%)	
Unclassified	12 (1.5%)	10 (1.5%)	2 (2.0%)	
Admission NIHSS, mean ± SD	2.0 (1.0–4.0)	2.0 (1.0–4.0)	6.0 (3.0–9.0)	<0.001
Admission mRS, mean ± SD	2.0 (1.0–3.0)	1.0 (1.0–3.0)	4.0 (3.0–4.0)	<0.001
Laboratory data				
White blood cell count, mean ±SD, 10^9 /L	7.1 ± 2.4	7.0 ± 2.2	8.0 ± 3.2	<0.001
Lymphocyte number, mean ±SD, 10^9 /L	1.7 ± 0.9	1.8 ± 0.9	1.4 ± 0.7	<0.001
Neutrophils number, mean ±SD, 10^9 /L	4.7 ± 2.2	4.6 ± 2.0	5.8 ± 3.0	<0.001
Monocyte number, mean ±SD, 10^9 /L	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.4	<0.001
Platelet count, mean ±SD, 10^9 /L	214.8 ± 78.2	215.3 ± 78.6	210.9 ± 76.2	0.331
Total cholesterol, mean ±SD, mmol/L	4.3 ± 1.3	4.4 ± 1.2	4.0 ± 1.2	0.009
HDL cholesterol, mean ±SD, mmol/L	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.7	0.021
Urea, mean ±SD, mmol/L	5.8 ± 2.5	5.7 ± 2.4	6.5 ± 2.9	0.006
Creatinine, mean ±SD, umol/L	81.4 ± 36.9	81.1 ± 36.9	83.3 ± 37.5	0.570
eGFRcr, mean ±SD, mL/min/1.73m²	80.4 ± 20.6	81.5 ± 20.0	72.7 ± 23.0	<0.001
eGFRcys, mean ±SD, mL/min/1.73m²	65.4 ± 22.3	65.5 ± 21.0	64.4 ± 29.5	0.658
eGFRcr-cys, mean ±SD, mL/min/1.73m²	69.7 ± 22.0	70.1 ± 20.5	67.2 ± 30.2	0.218

Abbreviations: TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, Large-Artery Atherosclerosis; CE, Cardioembolism; SAO, Small-Vessel Occlusion; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; HDL cholesterol, High-Density Lipoprotein cholesterol; eGFRcr, Estimated glomerular filtration rate calculated using serum creatinine; eGFRcys, Estimated glomerular filtration rate calculated using serum cystatin C; eGFRcr-cys, Estimated glomerular filtration rate calculated using both serum creatinine and cystatin C.

Statistical Tests of Cystatin C Levels in Relation to Stroke and 90-Day Outcomes

Statistical tests were conducted to evaluate the relationship between cystatin C levels and 90-day outcomes in the cohort (Table S1). Significant associations were identified through logistic regression analysis (P = 0.0023), MFP analysis (P =

0.0005), ANOVA (P = 0.0005), and quintile-based Chi-square testing (P < 0.00001). These results suggested that elevated cystatin C levels were linked to worse 90-day prognosis after AIS onset in the Stroke Center cohort.

Associations of Cystatin C with 90-Day Adverse Outcomes and Mortality

We evaluated the associations between cystatin C levels and 90-day adverse outcomes and mortality in the Stroke Center cohort. As shown in Table 2, the unadjusted model revealed a significant association between cystatin C and 90-day adverse outcomes (OR = 1.67, 95% CI: 1.20–2.31, p = 0.002) and mortality (HR = 1.84, 95% CI: 1.17–2.90, p = 0.008). These associations remained significant after adjusting for potential confounders. In Model I, which adjusted for gender and age, the odds ratio for adverse outcomes increased to 1.78 (95% CI: 1.28–2.49, p = 0.001), the hazard ratio for mortality rose to 2.05 (95% CI: 1.08–3.88, p = 0.027). Further adjustments in Model II (considering hypertension, diabetes, coronary heart disease, smoking, and drinking) and Model III (including lymphocyte count, total cholesterol, and eGFRcr) produced consistent results. The odds ratios for adverse outcomes were 1.69 (95% CI: 1.21–2.36, p = 0.002) and 2.13 (95% CI: 1.34–3.38, p = 0.001). The hazard ratios for mortality were 1.93 (95% CI: 1.10–3.37, p = 0.022) and 1.97 (95% CI: 1.09–3.54, p = 0.024). When cystatin C was analyzed as a categorical variable (higher vs lower levels), the group with elevated levels showed significantly increased risks for both adverse outcomes (OR = 1.64–2.05 across models, all p < 0.05) and mortality (HR = 1.82–2.05, all p < 0.05).

Mediating Effects of Inflammation Indexes

The study focused on four inflammatory indices as possible mediators between cystatin C levels and 90-day adverse outcomes following ischemic stroke: MLR, NLR, SIRI, and SII. After considering variables, the results showed that all four inflammatory indices considerably mediated the relationship between cystatin C and 90-day adverse outcomes. The mediation proportions were determined to be 18.7% (95% CI: 4.6%-68.1%, P = 0.010) for MLR, 20.2% (95% CI: 4.6%-79.2%, P = 0.006) for NLR, 16.1% (95% CI: 3.9%-65.4%, P = 0.006) for SIRI, and 17.4% (95% CI: 4.0%-63.8%, P = 0.006) for SII (as shown in Figure 3 and Table S2). These results indicated that inflammation played a crucial role in adverse outcomes.

Genetic Associations with Cystatin C and Ischemic Stroke

The MR study investigated the causal connection between cystatin C levels and the occurrence of ischemic stroke, particularly excluding cases of hemorrhagic stroke. Using a random effects model of the IVW method, the causal effect was evaluated. This choice was made because there was heterogeneity among the included studies, as shown by Cochran's Q test (P = 0.004 in IVW; P = 0.003 in MR Egger). IVW (OR = 1.10, 95% CI: 1.02–1.18, P = 0.011) and weighted median estimation (OR = 1.13, 95% CI: 1.03–1.25, P = 0.009) revealed a statistically significant association between genetically determined cystatin C levels and the risk of ischemic stroke. Although MR-Egger regression did not demonstrate a statistically significant association (OR = 1.10, 95% CI: 0.99–1.22, P = 0.070), the most important result in MR analysis is that of IVW. Further analyses, such as MR-Egger regression (intercept = −7.275017e - 06, P = 0.996),

Table 2 Association of Cystatin C with 90-Day Adverse Functional Outcomes and Mortality Calculated Using Regression Analysis and Cox Models in the Stroke Center Cohort

	Unadjusted		Model I		Model II		Model III	
90-day adverse functional outcomes	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Cystatin C (continuous)	1.67 (1.20,2.31)	0.002	1.78 (1.28,2.49)	0.001	1.69 (1.21,2.36)	0.002	2.13 (1.34,3.38)	0.001
Cystatin C								
Lower ≤1.15mg/L	1 (Ref.)		1 (Ref.)		1 (Ref.)		1 (Ref.)	
Higher >1.15mg/L	1.64 (1.07,2.51)	0.022	1.88 (1.21,2.90)	0.005	1.82 (1.17,2.83)	0.008	2.05 (1.16,3.61)	0.013
Mortality	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	Pvalue	HR (95% CI)	p value
Cystatin C (continuous)	1.84 (1.17,2.90)	0.008	2.05 (1.08,3.88)	0.027	1.93 (1.10,3.37)	0.022	1.97 (1.09,3.54)	0.024

Notes: The unadjusted model represented the univariate analysis. Cystatin C was associated with 90-day adverse outcomes (P = 0.002) and mortality (P = 0.008). Model I was adjusted for gender, age. Model II was adjusted for hypertension, diabetes, coronary heart disease, smoking and drinking based on Model I. Model III was further adjusted for lymphocyte number, total cholesterol count and eGFRcr based on Model II.

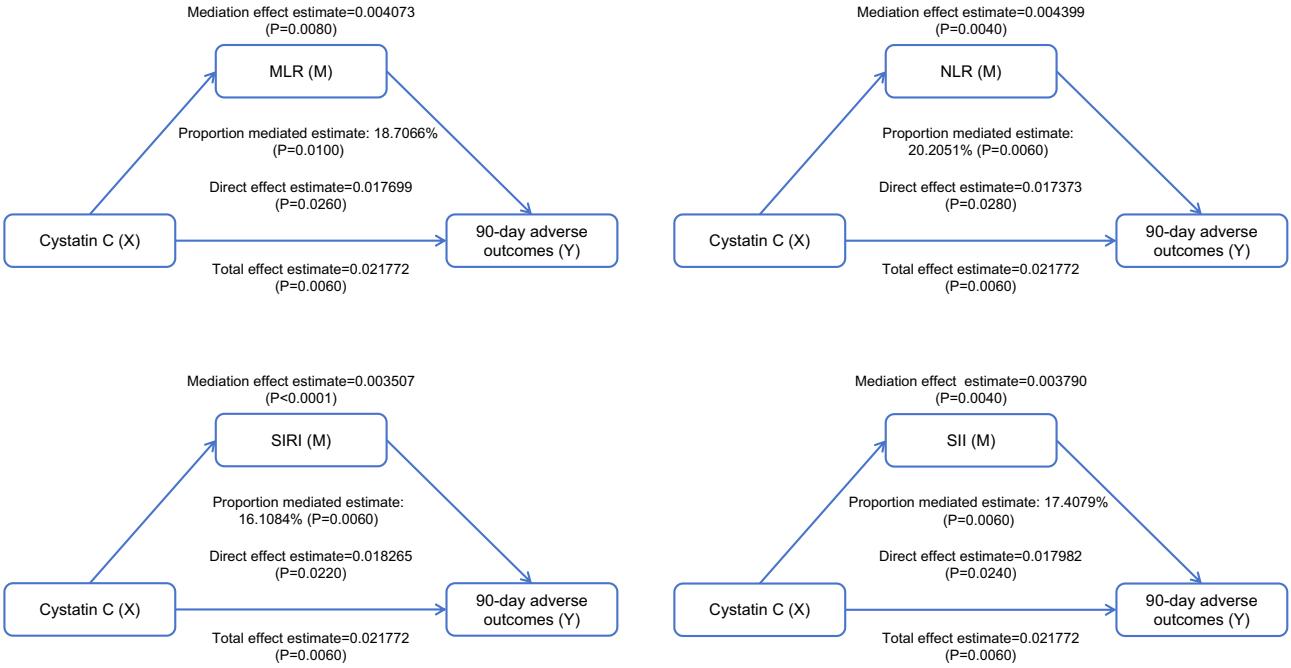

Figure 3 Inflammation indexes mediated the association of cystatin C with 90-day adverse outcomes in the Stroke Center cohort. Model was adjusted by gender, age, hypertension, diabetes, coronary heart disease, smoking and drinking.
Abbreviations: MLR, Monocyte-to-Lymphocyte Ratio; NLR, Neutrophil-to-Lymphocyte Ratio; SIRI, Systemic Inflammatory Response Index; SII, System Immune-Inflammation Index.

revealed no evidence of directional pleiotropy (Figure 4). The MR-PRESSO analysis did not find any outliers. Additionally, the leave-one-out analysis confirmed the robustness of the association, producing a B coefficient of 0.094 (P = 0.011). In conclusion, the MR analysis provided strong evidence in favor of a causal relationship between cystatin C levels and ischemic stroke.

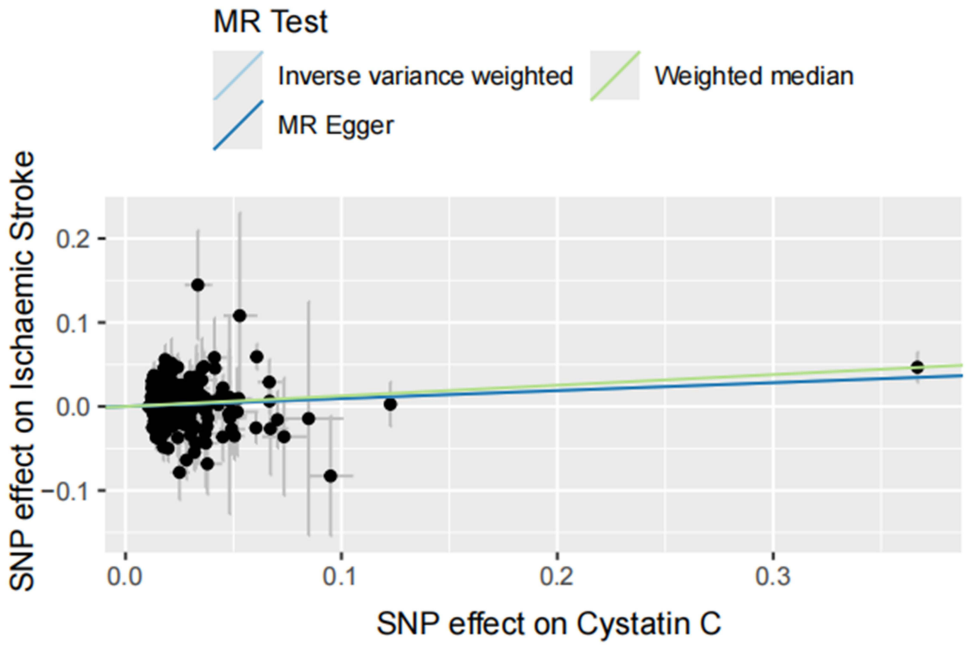

Figure 4 Scatter plot demonstrating a positive association between cystatin C and ischemic stroke in the MR analysis. A scatter plot was created based on the results of IVW (OR = 1.10, 95% CI: 1.02–1.18, P = 0.011), weighted median estimation (OR = 1.13, 95% CI: 1.03–1.25, P = 0.009) and MR-Egger (OR = 1.10, 95% CI: 0.99–1.22, P = 0.070).

Discussion

This study combined prospective cohort and Mendelian randomization (MR) analyses to investigate the role of cystatin C in ischemic stroke. Elevated serum cystatin C levels were independently linked to poor 90-day functional outcomes and mortality in patients with AIS. Systemic inflammation, assessed through MLR, NLR, SIRI, and SII, mediated 16% to 20% of this association, highlighting a significant pathological pathway. MR analyses using 290 genetic instruments provided robust evidence for a causal link between genetically predicted higher cystatin C levels and increased ischemic stroke risk (OR = 1.10, 95% CI: 1.02–1.18, p=0.011). Cystatin C may serve as a valuable integrated biomarker for prognosticating outcomes in AIS and predicting the risk of ischemic stroke.[24] Additionally, it may act as a potential mediator that links renal dysfunction, inflammation, and cerebrovascular injury.[25,26]

The observation of cystatin C levels increasing significantly within 24 hours of stroke onset, preceding changes in serum creatinine, underscores its value as a sensitive marker.[27] This timing difference likely arises from the unique characteristics of cystatin C. As a low-molecular-weight protein produced by all nucleated cells, cystatin C is less influenced by factors such as age, muscle mass, or diet compared to creatinine.[28] Its shorter half-life allows for quicker detection of physiological stress, while creatinine's delayed response is due to its reliance on muscle metabolism.[29,30]

This acute rise in cystatin C is likely linked to stroke-induced neurohumoral activation, where sympathetic overdrive and systemic inflammation disrupt renal homeostasis through two main pathways.[31,32] On one hand, these factors directly impair renal hemodynamic autoregulation and tubular reabsorption.[33] On the other hand, activation of the RAS worsens vasoconstriction and endothelial injury.[34] These changes can affect renal function before significant alterations in creatinine levels are observed. Consequently, the rise in cystatin C not only indicates a decline in glomerular filtration rate but may also contribute to a cycle of blood pressure dysregulation through increased angiotensin II production.[35] This underscores its role in identifying subclinical renal injury early and highlights potential mechanisms linking renal stress to cerebrovascular risk, particularly relevant in contexts involving blood pressure dysregulation.

Beyond these acute responses, chronic renal impairment correlated strongly with poor outcomes. Chronic kidney disease, recognized as an independent risk factor for stroke, may increase the risk of cerebrovascular events through several mechanisms.[36] Mechanistically, as supported by prior research, the accumulation of uremic toxins can activate inflammatory cells, releasing pro-inflammatory factors potentially destabilizing atherosclerotic plaques.[37] Furthermore, cystatin C itself may impair the endothelial barrier by inhibiting cysteine protease activity, affecting vasodilation and promoting thrombosis.[38,39] Deteriorating renal function also reduces antioxidant capacity, increasing reactive oxygen species accumulation which can compromise the blood-brain barrier integrity and worsen ischemic penumbral injury.[40]

The finding that systemic inflammation mediated 16–20% of the association between cystatin C and poor outcomes further highlights the complex interaction between renal dysfunction and cerebrovascular injury. The findings further solidify cystatin C's dual role: as a sensitive biomarker of glomerular filtration function and a potential indicator/mediator of inflammatory vascular injury pathways impacting cerebrovascular health. The MR analysis provided evidence for causality (OR=1.10, 95% CI: 1.02–1.18, p=0.011), suggesting that elevated cystatin C levels may directly contribute to ischemic stroke pathogenesis through pathways potentially extending beyond classical renal dysfunction.[41]

Our findings are consistent with and expand upon previous epidemiological studies. The strong association between elevated cystatin C levels and poor stroke outcomes reinforces results from the NHANES cohort, which identified a nonlinear relationship with stroke morbidity and established a critical threshold at 1.24 mg/L.[14] The prognostic significance of cystatin C is further supported by its inclusion in biomarker-enhanced risk scores, such as the ABC-bleeding score for patients with atrial fibrillation.[42,43] Our study provides genetic evidence for causality through Mendelian randomization and quantifies the mediating role of systemic inflammation (16–20%). These findings suggest that cystatin C could serve as a valuable clinical biomarker for guiding personalized management strategies in acute ischemic stroke, potentially aiding in risk stratification and targeted interventions, such as controlling inflammation, to improve patient outcomes.

However, several limitations should be considered. First, in the management of acute stroke, routine assessments of renal function are prioritized, while cystatin C measurement is not typically included in emergency protocols. As a result, many patients lacked dynamic cystatin C data during the 48-hour follow-up, which may have introduced selection bias and obscured

early renal fluctuations that could affect outcome associations. Second, as an observational cohort study conducted in an East Asian population, the findings may be influenced by residual confounding from factors such as body weight, dietary habits, ethnic characteristics and other unmeasured variables. Third, although Mendelian randomization analyses suggested a causal relationship between cystatin C and stroke risk, the possibility of residual pleiotropy among genetic instruments cannot be entirely ruled out, despite thorough sensitivity analyses. Future studies should incorporate cystatin C into standard kidney function assessments for acute ischemic stroke, confirm its predictive value in larger multi-ethnic groups, and explore targeted therapies to modify cystatin C-related pathways for better clinical outcomes.

Conclusion

This study demonstrates that elevated cystatin C is not only a causal risk factor for ischemic stroke but also an independent predictor of worse 90-day neurological function and higher mortality following acute ischemic stroke. Furthermore, the relationship between cystatin C and poor functional prognosis is partially mediated by systemic inflammatory biomarkers (MLR, NLR, SIRI and SII).

Data Sharing Statement

The Stroke Center cohort data are available from the corresponding authors (Minghua Wu: yfy0069@njucm.edu.cn; Yuan Zhu: zy1987424@163.com) upon reasonable request. The GWAS datasets are publicly accessible.

Ethical Approval

The cohort study at the Stroke Center received approval from the Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine, in accordance with the ethical guidelines of the Helsinki Declaration (2017NL-012-01). Before enrollment, all participants in the cohort completed the informed consent process to ensure their voluntary participation.

Acknowledgments

In the study, the Stroke Center cohort conducted at Jiangsu Province Hospital of Chinese Medicine.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by Natural Science Foundation of China (Grant No. 82274428, 82474435) from Minghua Wu and Wenlei Li, Jiangsu Natural Science Foundation (BK20241996) from Wenlei Li, Jiangsu Province Administration of Chinese Medicine (ZT202102) from Minghua Wu, Key Medical Research Project of Jiangsu Provincial Health Commission (K2023009) from Zhaoyao Chen.

Disclosure

The authors declare no competing interests in this work.

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