



Association between high-sensitivity C-reactive protein, functional disability, and stroke recurrence in patients with acute ischaemic stroke: A mediation analysis

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Summary

Background Post-stroke inflammation biomarker high-sensitivity C-reactive protein (hsCRP) increases cerebral infarct size and results in functional disability directly, it also contributes to the formation and maturation of atherosclerotic plaques, which increase the risk of stroke recurrence and results in functional disability indirectly. However, no study has quantified how much functional disability was mediated by stroke recurrence.

Methods Patients with acute ischaemic stroke within 7 days and admitted to 169 hospitals in the Third China National Stroke Registry were analyzed. Blood samples were collected within 24 h of admission. Stroke recurrence and functional disability (defined as a modified Rankin scale score ≥ 2) were assessed via face-to-face interviews at three months. Mediation analysis under the counterfactual framework was performed to examine the potential causal chain in which stroke recurrence may mediate the relationship between hsCRP and functional outcome. Sensitivity analyses were performed across different subgroups and on different scales of hsCRP measurement.

Findings Of the 7603 analyzed patients (mean [SD] age, 62.3 [11.3] years; 2392 [31.5%] women), the median (interquartile range [IQR]) of NIHSS score was 3.0 (1.0–6.0). The median (IQR) level of hsCRP was 1.73 (0.81–4.38) mg/L. A total of 496 (6.5%) cases of stroke recurrence and 1884 (24.8%) cases of functional disability were observed at the 90-day follow-up. Each SD increase in the concentration of hsCRP was associated with an increased risk of stroke recurrence (adjusted odds ratio [aOR], 1.11; 95% CI, 1.04–1.18) and disability (aOR, 1.14; 95% CI, 1.08–1.20) within 90 days. Of 1884 functionally disabled patients, only 16.0% ($n = 302$) of patients experienced stroke recurrence before functional disability. Stroke recurrence during follow-up explained 16.52% (95% CI, 5.79%–27.25%) of the relationship between hsCRP and functional disability. Sensitivity analyses in different subgroups and on different scales of hsCRP measurement showed comparable results.

Interpretation Stroke recurrence mediates less than 20% of the association between hsCRP and functional disability at 90 days among patients with acute ischaemic stroke. In addition to typical secondary prevention strategies for preventing stroke recurrence, more attention should be paid to novel anti-inflammatory therapy to improve functional outcomes.

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Research in context

Evidence before this study

We searched PubMed to identify relevant publications published up to Dec 31, 2021, on the association between high-sensitivity C-reactive protein (hsCRP), functional disability, and stroke recurrence for patients with ischaemic stroke using the terms “C-reactive protein” and (“ischaemic stroke” OR “ischemic stroke” OR “cerebral infarction” OR “cerebral infarct”), without language restrictions. We identified several studies that assessed the association of hsCRP with stroke recurrence or functional disability, and stroke recurrence with functional disability. However, no study hypothesized and assessed the mediation effect of stroke recurrence between the link of hsCRP and functional disability.

Added value of this study

In this cohort study of 7603 patients with acute ischaemic stroke, concentration of hsCRP was found to be associated higher risk of stroke recurrence and functional disability at 90 days as well. However, stroke recurrence during follow-up explained less than 20% of the association between high-sensitivity C-reactive protein and functional disability.

Implications of all evidence available

These findings suggest that typical secondary prevention strategies for preventing stroke recurrence are far from sufficient to improve the functional outcomes for patients with acute ischaemic stroke, and novel anti-inflammatory therapy should be paid more attention.

Introduction

Inflammation plays a critical role in the pathogenesis and prognosis of ischaemic stroke.^{1,2} Acute-phase protein C-reactive protein (CRP), or high-sensitivity c-reactive protein (hsCRP, CRP measured with a high-sensitivity assay), is a nonspecific biomarker of inflammation, which is reported to be positively associated with higher risks of stroke recurrence and functional damage for stroke survivors as well.^{3–6} Evidence also showed that stroke recurrence was highly associated with functional disability.^{7,8} Biologically, on the one hand, post-stroke inflammation biomarker hsCRP would cause cell death, brain injury, and blood-brain barrier disruption, which result in functional damage directly.^{9–12} But on the other hand, it contributes to atherosclerosis, plaque rupture, platelet aggregation, and intravascular thrombosis, which

cause stroke recurrence first and then result in functional damage indirectly.¹³ However, it remains unclear to what extent, stroke recurrence mediates the link between the high level of hsCRP and functional disability.

We hypothesized that the relationship between hsCRP and functional outcomes at 90 days is mediated by follow-up stroke recurrence. Using data from the Third China National Stroke Registry (CNSR-III), we investigated to what extent, if any, stroke recurrence mediated the relationship between hsCRP and functional outcomes at 90 days among patients with acute ischaemic stroke and assessed the mediation effect in key important subgroups and on different scales of hsCRP.

Methods

This study followed A Guideline for Reporting Mediation Analyses of Randomized Trials and Observational Studies (AGReMA) reporting guideline.¹⁴

Study design and participants

Data for this study were derived from the Third China National Stroke Registry (CNSR-III), a large-scale nationwide prospective registry of acute ischaemic cerebrovascular events in China. The study design and patient enrollment for the CNSR-III were reported previously.¹⁵ In brief, the CNSR-III enrolled 15,166 consecutive patients with ischaemic stroke and transient ischaemic attack (TIA) from 201 hospitals in China between August 2015 and March 2018. Patients who were over 18 years old and had suffered acute ischaemic stroke or TIA with a symptom onset within seven days were enrolled in this registry. Acute ischaemic stroke was diagnosed according to the World Health Organization criteria with confirmation by brain magnetic resonance imaging (MRI) or computed tomography (CT).¹⁶

Of 201 participating hospitals, 169 hospitals participated in both the prespecified biomarker substudy and the imaging substudy. Therefore, we included only patients from these 169 sites in this analysis. TIA patients, ischaemic stroke patients with prior mRS scores ≥ 2 , patients who had undergone intravenous thrombolysis or thrombectomy, and patients with missing data on hsCRP or 3-month mRS scores were excluded from this analysis.

Data collection

Trained neurologists from participating hospitals collected baseline data through face-to-face interviews

according to a standard operating protocol. Baseline data included demographics (sex, age, body mass index); National Institutes of Health Stroke Scale (NIHSS) score; pre-stroke modified Rankin scale score; blood pressure; smoking status; and medical history, including prior stroke or TIA, hypertension, diabetes mellitus, dyslipidemia, coronary heart disease or myocardial infarction, and atrial fibrillation.

Blood samples were collected within 24 h of admission (median time of 55 h after index event onset). Plasma specimens were extracted, aliquoted and transported through cold chain to the central laboratory in Beijing Tiantan Hospital and stored at -80°C until tests were performed centrally and blindly. The concentration of hsCRP was determined by using enzyme-linked immunosorbent assay kits (catalogue number: PHS600C, R&D Systems, Inc, Minneapolis, MN, USA).

Imaging data, including brain MRI (T_1 -weighted, T_2 -weighted, fluid-attenuated inversion recovery [FLAIR], diffusion-weighted imaging [DWI] with apparent diffusion coefficient [ADC] maps, magnetic resonance angiography [MRA], T_2^* /SWI) or CT (if there was any contraindication to MRI), were collected in Digital Imaging and Communications in Medicine (DICOM) format on discs and sent to the imaging research center of Beijing Tiantan Hospital. Brain imaging, at least one vascular assessment of intracranial and extracranial arteries, and heart examination were performed at baseline following standard protocols. Acute infarction was diagnosed according to the presence of hyperintensity on DWI and was further classified as single acute infarction (uninterrupted lesions visible in contiguous territories), multiple acute infarctions (more than one topographically distinct lesion) or watershed infarction.¹⁷ The etiology classification were large-artery atherosclerosis (LAA), cardioembolism (CE), small-artery occlusion (SAO), other determined cause, or undetermined cause according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria.¹⁸

Patient follow-up and outcomes

Three months after initial data collection, patients were followed up by trained research coordinators in face-to-face interviews based on a standardized interview protocol. Stroke recurrence was defined as a new neurological deficit lasting more than 24 h or rehospitalization with a diagnosis of ischaemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage. Functional outcomes were measured by the modified Rankin scale (mRS range of 0–6, with a score of 0 indicating no disability and higher scores indicating more severe disability); Functional disability was defined as an mRS score ≥ 2 , as approximately half of the ischaemic strokes were minor strokes in the CNSR-III registry.

Statistical analysis

Baseline characteristics were described and compared by quantiles of hsCRP. Continuous variables that followed a normal distribution were reported as the means and standard deviations and tested by analysis of variance (ANOVA); those that were not normally distributed were reported as medians and interquartile ranges (IQRs) and compared using Kruskal–Wallis tests. Categorical variables were presented as frequencies and percentages and were tested using chi-square tests. A SAS macro called %ggBaseline was used to analyze and report the baseline characteristics automatically.¹⁹ Associations between hsCRP and stroke recurrence or disability at 90 days were measured by odds ratios (ORs) and 95% CIs based on logistic regression models. The concentration of hsCRP was first treated as a categorical variable with Q_1 as the reference group; next, it was treated as a continuous variable, with increments of 1 standard deviation, in logistic models.

To explicate the association of hsCRP with functional outcome, indirect associations acting through stroke recurrence as a mediating variable and direct associations not mediated by stroke recurrence were quantified (Figure 1). We performed a causal mediation analysis under a counterfactual framework that provides a general framework offering clear definitions of causal mediation and related effects.^{20–22} Under this framework, the total effect (TE) could be decomposed into two components: the natural direct effect (NDE) and the natural indirect effect (NIE); each was measured as an odds ratio (OR). The NDE represented the effect of hsCRP on functional disability that was independent of stroke recurrence. An NIE represented the effect of hsCRP on functional disability that could be explained by changes in status of follow-up stroke recurrence. The mediation effect is measured by percentage mediated (PM), computed as $\text{NIE}/\text{TE} \times 100\%$ on a log-transformed odds ratio scale, which is the percentage of the total effect that is mediated by the mediator.²³ Under this framework, two logistic regression models were fitted: a multivariate logistic regression model for stroke recurrence (mediator) conditional on hsCRP (exposure) and all study confounders and another multivariate logistic regression model for functional disability (outcome) conditional on hsCRP, stroke recurrence, and all study confounders. Factors known to be associated with stroke recurrence and functional outcomes were included in the analyses as confounders, which included demographics (age, sex, body mass index), NIHSS score at admission, smoking status, systolic blood pressure, diastolic blood pressure, medical history (prior stroke/TIA, hypertension, diabetes mellitus, prior coronary heart disease/myocardial infarction, atrial fibrillation/flutter), image data (infarction pattern, infarction location) and TOAST subtypes.

All p -values were two-sided, with $p < 0.05$ considered statistically significant. All statistical analyses were

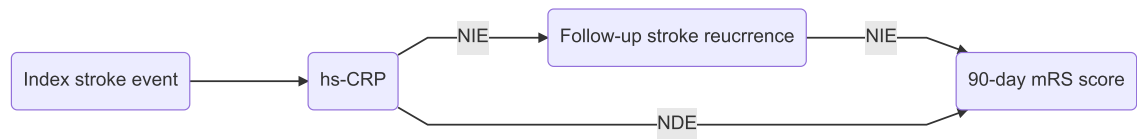


Figure 1. Illustration of mediation effect. mRS, indicates modified Rankin scale., natural direct effect; NIE, natural indirect effect. Total effect = NDE + NIE.

performed using SAS V.9.4 (SAS Institute Inc., Cary, NC, USA).

Sensitivity analyses

To test the robustness of our analysis, we performed a series of sensitivity analyses. First, we recalculated the mediation effect after excluding cases died before stroke recurrence to avoid competing risks of death. Second, we performed causal mediation analysis stratified by admission time (≤ 6 h, > 6 h, or unknown), NIHSS score (> 3 , ≤ 3), infarction pattern, infarction location, and TOAST subtype, as functional outcomes may be affected by stroke severity, infarction pattern, infarction location, and subtype of ischaemic stroke. Third, we assessed the association of hsCRP with stroke recurrence and disability and estimated the mediated effect of stroke recurrence on the original-scale, log-scale, and per SD of log-scale measurements of hsCRP concentration.

Ethics statement

The study was approved by the ethics committee of Beijing Tiantan Hospital (KY2015-001-01). Written informed consent was obtained from each participant or his/her representative before data collection.

Role of the funding source

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Results

A total of 11384 acute ischaemic stroke patients with prior mRS < 2 who did not receive thrombolysis or thrombectomy therapy were included in the initial assessment. After the further exclusion of 4432 patients without blood or imaging samples, had missing data on hsCRP, or lost to follow-up, and additional inclusion of 651 patients with hsCRP data imputed from local centers, a total of 7603 patients were included in this analysis (Figure S1 in the Supplement). Patients included in and excluded from this analysis were largely comparable (Table S1 in the Supplement).

Of the 7603 patients analyzed, the mean [SD] age was 62.3[11.3] years old; 2392 (31.5%) were women, and the median NIHSS score at admission was 3.0 (interquartile range 1.0–6.0). The most common prior medical condition was hypertension (62.9% [$n = 4785$]), followed by diabetes mellitus (24.5% [$n = 1859$]) and prior stroke/TIA (22.2% [$n = 1691$]). Nearly one-half of the patients were single infarction (46.6% [$n = 3542$]), and the other half were multiple infarctions (45.3% [$n = 3442$]). Over 56% ($n = 4289$) of patients were infarcts in the anterior circulation. One-quarter of patients had LAA, and another quarter had SAO (Table 1).

Baseline characteristics by quartile of hsCRP

The median hsCRP concentration was 1.73 mg/L (interquartile range 0.81–4.38). Detailed distribution of hsCRP was displayed in Figure S2 in the Supplement. Compared to patients with the lowest quartile of hsCRP, patients with the highest quartile of hsCRP were older (65.1 ± 11.5 vs 60.7 ± 11.0 , F test $P < .001$) and had higher NIHSS scores at admission (median [IQR], 4.0 [2.0–7.0] vs 3.0 [1.0–5.0], Kruskal–Wallis test $P < .001$); and a higher prevalence of prior stroke/TIA (470 [24.7%] vs 418 [22.2%], chi-square test $P = 0.018$), hypertension (1252 [65.8%] vs 1100 [58.5%], chi-square test $P < .001$), diabetes (496 [26.1] vs 415 [22.1], chi-square test $P < .001$), dyslipidemia (154 [8.1%] vs 130 [6.9%], chi-square test $P = 0.017$), CHD/MI (233 [12.3%] vs 143 [7.6%], chi-square test $P < .001$), and atrial fibrillation (207 [10.9%] vs 67 [3.6%], chi-square test $P < .001$). A higher quartile of hsCRP was also positively associated with a higher prevalence of multiple infarctions (1042 [54.8%] vs 732 [38.9%], chi-square test $P < .001$), simultaneous anterior and posterior circulatory infarctions (161 [8.5%] vs 97 [5.2%], chi-square test $P < .001$), and the LAA subtype of ischaemic stroke (607 [31.9%] vs 378 [20.1%], chi-square test $P < .001$) (Table 1).

Association between hsCRP, stroke recurrence and disability

A total of 496 (6.5%) and 1884 (24.8%) patients experienced stroke recurrence and functional disability respectively during 90-day follow-up, respectively. Compared to patients with hsCRP levels in the lowest quartile,

Variables	Total (N = 7603)	Quartile 1 (N = 1881)	Quartile2 (N = 1916)	Quartile3 (N = 1904)	Quartile4 (N = 1902)	P Value
hsCRP level, mg/L	1.73 (0.81–4.38)	>0.81	0.81–1.73	1.73–4.38	>4.38	
Demographic						
Age	62.3 ± 11.3	60.7 ± 11.0	61.2 ± 10.8	62.3 ± 11.5	65.1 ± 11.5	<.001
Women	2392 (31.5)	518 (27.5)	593 (30.9)	655 (34.4)	626 (32.9)	<.001
Smoking	2431 (32.0)	638 (33.9)	637 (33.2)	594 (31.2)	562 (29.5)	0.016
BMI	24.7 ± 3.3	24.2 ± 3.0	24.9 ± 3.2	25.1 ± 3.5	24.8 ± 3.5	<.001
NIHSS score at admission	3.0 (1.0–6.0)	3.0 (1.0–5.0)	3.0 (1.0–5.0)	3.0 (2.0–5.0)	4.0 (2.0–7.0)	<.001
Blood pressure, mmHg						
SBP	151.0 ± 22.5	149.9 ± 22.1	150.8 ± 22.4	152.7 ± 22.4	150.6 ± 23.0	<.001
DBP	87.7 ± 13.3	87.6 ± 13.0	87.8 ± 12.8	88.6 ± 13.6	86.7 ± 13.6	<.001
Medical history						
Prior Stroke/TIA	1691 (22.2)	418 (22.2)	406 (21.2)	397 (20.9)	470 (24.7)	0.018
Hypertension	4785 (62.9)	1100 (58.5)	1209 (63.1)	1224 (64.3)	1252 (65.8)	<.001
Diabetes mellitus	1859 (24.5)	415 (22.1)	429 (22.4)	519 (27.3)	496 (26.1)	<.001
Dyslipidemia	594 (7.8)	130 (6.9)	133 (6.9)	177 (9.3)	154 (8.1)	0.017
Prior CHD/MI	737 (9.7)	143 (7.6)	160 (8.4)	201 (10.6)	233 (12.3)	<.001
Atrial fib/flutter	468 (6.2)	67 (3.6)	95 (5.0)	99 (5.2)	207 (10.9)	<.001
Infarction pattern						
None	494 (6.5)	129 (6.9)	143 (7.5)	118 (6.2)	104 (5.5)	
Single infarction	3542 (46.6)	996 (53.0)	954 (49.8)	872 (45.8)	720 (37.9)	
Multiple infarction	3442 (45.3)	732 (38.9)	796 (41.5)	872 (45.8)	1042 (54.8)	
Watershed infarction	125 (1.6)	24 (1.3)	23 (1.2)	42 (2.2)	36 (1.9)	
Infarction circulation						
None	494 (6.5)	129 (6.9)	143 (7.5)	118 (6.2)	104 (5.5)	<.001
Anterior circulating infarction	4289 (56.4)	1036 (55.1)	1068 (55.7)	1086 (57.0)	1099 (57.8)	
Posterior circulation infarction	2332 (30.7)	619 (32.9)	600 (31.3)	575 (30.2)	538 (28.3)	
Anterior and posterior circulatory infarction	488 (6.4)	97 (5.2)	105 (5.5)	125 (6.6)	161 (8.5)	
TOAST						
LAA	1998 (26.3)	378 (20.1)	481 (25.1)	532 (27.9)	607 (31.9)	<.001
CE	420 (5.5)	78 (4.1)	103 (5.4)	88 (4.6)	151 (7.9)	
SAO	1930 (25.4)	608 (32.3)	521 (27.2)	465 (24.4)	336 (17.7)	
Other	3255 (42.8)	817 (43.4)	811 (42.3)	819 (43.0)	808 (42.5)	

Table 1: Demographic and clinical characteristics of patients by quartile of hsCRP at admission.

Abbreviations: hsCRP indicates high sensitivity C-reactive protein; BMI, body mass index; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure; TIA, transient ischaemic attack; CHD, coronary heart disease; MI, myocardial infarction; TOAST, the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria; LAA, large-artery atherosclerosis; CE, cardioembolism; and SAO, small-artery occlusion.

those with highest quartile of hsCRP tended to have a higher risk of stroke recurrence (8.2% vs. 5.3%; adjusted odds ratio [aOR] 1.26, 95% CI, 0.96–1.65) and a significantly higher risk of disability (36.5% vs. 18.6%; aOR 1.55, 95% CI, 1.31–1.83) at 90 days. In addition, a 1-SD increase in hsCRP was associated with a 11% increase of the adjusted risk of stroke recurrence (aOR 1.11, 95% CI 1.04–1.18) and a 14% increase of the adjusted risk of disability (aOR 1.14, 95% CI 1.08–1.20) at 90 days (Table 2).

Mediation effect of stroke recurrence

Of 1884 functionally disabled patients, 16.0% ($n = 302$) of patients experienced stroke recurrence before

disability. The total, direct associations, and indirect associations of hsCRP with functional disability were presented in Table 3. The indirect association via follow-up stroke recurrence implied that a 2% increase in risk of functional disability (aOR 1.02; 95% CI, 1.01–1.04) would be observed on average. The proportion of the association between hsCRP and functional disability mediated by stroke recurrence was 16.52% (95% CI, 5.79–27.25%). We also calculated the estimates of direct and indirect associations among patients excluding death patients before stroke recurrence to avoid the influence of competing risk of death. Results showed that stroke recurrence mediated 20.91% (95% CI 7.40–34.41%) of the association between hsCRP and functional disability (Table 3).

Outcomes	No of patients	Event (%)	Crude Analysis		Adjusted Analysis	
			Crude OR (95% CI)	Crude P	Adjusted OR (95% CI)	Adjusted P
Stroke recurrence at 90 day						
Quartile 1	1881	99 (5.3)	1.00 (Reference)		1.00 (Reference)	
Quartile 2	1916	116 (6.1)	1.16 (0.88–1.53)	0.29	1.09 (0.82–1.44)	0.55
Quartile 3	1904	125 (6.6)	1.26 (0.96–1.66)	0.090	1.10 (0.83–1.45)	0.50
Quartile 4	1902	156 (8.2)	1.61 (1.24–2.09)	<.001	1.26 (0.96–1.65)	0.097
Per SD			1.14 (1.07–1.21)	<.001	1.11 (1.04–1.18)	0.001
Disability at 90 day (mRS>=2)						
Quartile 1	1881	349 (18.6)	1.00 (Reference)		1.00 (Reference)	
Quartile 2	1916	375 (19.6)	1.07 (0.91–1.26)	0.42	1.02 (0.85–1.22)	0.84
Quartile 3	1904	466 (24.5)	1.42 (1.22–1.66)	<.001	1.15 (0.97–1.37)	0.11
Quartile 4	1902	694 (36.5)	2.52 (2.17–2.93)	<.001	1.55 (1.31–1.83)	<.001
Per SD			1.27 (1.20–1.34)	<.001	1.14 (1.08–1.20)	<.001

Table 2: Association between hsCRP and stroke recurrence and functional outcome at 90 days.

Abbreviations: hsCRP indicates high sensitivity C-reactive protein; OR, odds ratio; and SD, standard deviation.

*Adjusted for demographics (age, sex, body mass index), the National Institutes of Health Stroke Scale score at admission, smoking status, systolic blood pressure, diastolic blood pressure, medical history (prior stroke/ transient ischemic attack, hypertension, diabetes mellitus, dyslipidemia, prior coronary heart disease / myocardial infarction, atrial fib/flutter), and image data (infarction pattern, infarction location) and etiology classification.

Sensitivity analysis of mediation analysis

Data across major strata defined by admission time, NIHSS score at admission, infarction pattern, infarction location, and etiological subtype showed comparable results (Figure 2). Sensitivity analyses on the log-scale, and per SD of log-scale hsCRP concentration showed that hsCRP was associated with stroke recurrence and functional outcomes as well (Table S2 in the Supplement), and the mediation effects were consistent with the primary analysis (percentage mediated, 14.00% [95% CI, 3.51–24.50%] on the log-scale and 13.11% [95% CI, 3.20–23.02%] on per SD of log-scale of hsCRP, respectively) (Table 4).

Discussion

In this multicenter cohort study, our mediation analysis showed that stroke recurrence only partially mediated the relationship between hsCRP and functional outcomes at 90 days. Less than 20% of the variance in functional outcomes as captured by mRS scores could be attributed to a difference in stroke recurrence, suggesting that typical secondary prevention strategies for preventing stroke recurrence are far from sufficient to improve functional independence, and novel anti-inflammatory therapy should be paid more attention for this population.

Previous reports yield inconsistent results on whether hsCRP was associated with stroke recurrence

Effect	Unadjusted analysis		Adjusted analysis*	
	Estimate (95% CI)	P	Estimate (95% CI)	P
Of 7603 patients				
Total Effect (TE), Odds Ratio	1.29 (1.21–1.36)	<.001	1.16 (1.09–1.23)	<.001
Natural Direct Effect (NDE), Odds Ratio	1.25 (1.18–1.32)	<.001	1.13 (1.07–1.20)	<.001
Natural Indirect Effect (NIE), Odds Ratio	1.03 (1.01–1.04)	<.001	1.02 (1.01–1.04)	0.002
Percentage Mediated (PM)	11.59 (5.99–17.19)	<.001	16.52 (5.79–27.25)	0.003
Of 7542 patients †				
Total Effect (TE), Odds Ratio	1.26 (1.19–1.33)	<.01	1.14 (1.07–1.21)	<.001
Natural Direct Effect (NDE), Odds Ratio	1.22 (1.16–1.29)	<.0001	1.11 (1.04–1.18)	0.001
Natural Indirect Effect (NIE), Odds Ratio	1.03 (1.02–1.04)	<.001	1.03 (1.01–1.04)	0.001
Percentage Mediated (PM)	14.06 (7.59–20.53)	<.001	20.91 (7.40–34.41)	0.002

Table 3: Proportion of association of per SD of hs-CRP with 90-day mRS mediated by follow-up stroke recurrence.

Abbreviations: SD indicates standard deviation, hs-CRP, high-sensitivity C-reactive protein; mRS, modified Ranking scale.

*Adjusted for demographics (age, sex, body mass index), the National Institutes of Health Stroke Scale score at admission, smoking status, systolic blood pressure, diastolic blood pressure, medical history (prior stroke/ transient ischemic attack, hypertension, diabetes mellitus, dyslipidemia, prior coronary heart disease / myocardial infarction, atrial fib/flutter), and image data (infarction pattern, infarction location) and etiology classification.

† 61 deaths without stroke recurrence were excluded.

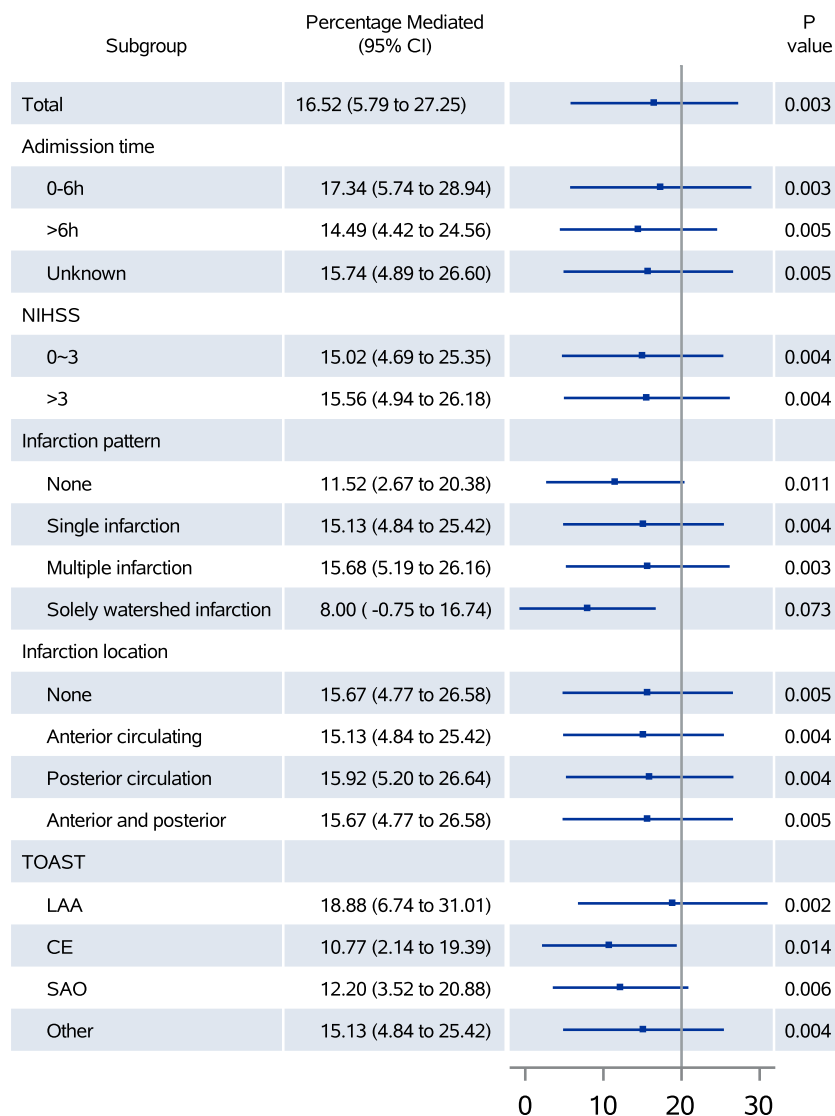


Figure 2. Causal mediation analysis stratified by prespecified subgroup NIHSS indicates National Institutes of Health Stroke Scale; LAA, large-artery atherosclerosis; CE, cardioembolism; SAO, small-artery occlusion.

or functional outcomes after ischaemic stroke. For instance, evidence from a subgroup analysis of CHANCE (Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events) trial indicated that elevated hsCRP was associated with both higher risk of recurrent stroke and poor function outcome.³ Data from a systematic review and meta-analysis showed the association of hsCRP with stroke recurrence as well,²⁴ and results from Song et al. also suggested that hsCRP concentration was a predictor of functional disability after first-ever stroke.⁶ However, the NORTHSTAR (North West of England transient ischaemic attack and minor stroke) study and a study by Elkind et al. showed that hsCRP was not associated with stroke recurrence.^{25,26} The mixed results may due to

substantial methodological heterogeneity between studies. Therefore, individual patient data pooled meta-analysis would produce more reliable evidence. Although no such evidence is available yet, an individual participant meta-analysis among stroke-free population confirmed the nearly log-linear relationship between hsCRP level and risk of incident stroke.²⁷

Similar to the subgroup analysis of the CHANCE study,³ we observe that hsCRP were both positively associated with stroke recurrence and functional disability in our study. In addition, from a biological view,²⁸⁻³⁰ acute ischaemic stroke event would result in neuronal damage and functional disability, which had also been indicated by data from previous reports in clinical practice.^{7,8} As thus, we treated stroke recurrence as a

Variables	Unadjusted analysis		Adjusted analysis*	
	Estimate (95%CI)	P	Estimate (95%CI)	P
Log-scale hsCRP				
Odds Ratio Total Effect	2.08 (1.87–2.28)	<.001	1.45 (1.29–1.62)	<.001
Odds Ratio Natural Direct Effect (NDE)	1.94 (1.76–2.11)	<.001	1.39 (1.24–1.54)	<.001
Odds Ratio Natural Indirect Effect (NIE)	1.07 (1.04–1.11)	<.001	1.05 (1.01–1.08)	0.018
Percentage Mediated	13.11 (7.55–18.68)	<.001	14.00 (3.51–24.50)	0.009
Per SD of log-scale hsCRP				
Odds Ratio Total Effect	1.52 (1.44–1.61)	<.001	1.24 (1.16–1.32)	<.001
Odds Ratio Natural Direct Effect (NDE)	1.46 (1.39–1.54)	<.001	1.21 (1.14–1.28)	<.001
Odds Ratio Natural Indirect Effect (NIE)	1.04 (1.02–1.06)	<.001	1.03 (1.00–1.05)	0.016
Percentage Mediated	11.56 (6.61–16.51)	<.001	13.11 (3.20–23.02)	0.001

Table 4: Proportion of association of hsCRP with 90-day mRS mediated by follow-up stroke recurrence on different scale of hsCRP.

Abbreviations: hs-CRP indicates high-sensitivity C-reactive protein; mRS, modified Ranking scale.

*Adjusted for demographics (age, sex, body mass index), the National Institutes of Health Stroke Scale score at admission, smoking status, systolic blood pressure, diastolic blood pressure, medical history (prior stroke/ transient ischemic attack, hypertension, diabetes mellitus, dyslipidemia, prior coronary heart disease / myocardial infarction, atrial fib/flutter), and image data (infarction pattern, infarction location) and etiology classification.

mediator of the link between hsCRP and functional disability and estimated the mediation effect via mediation analysis. To the best of our knowledge, no report has hypothesized and tested the mediation effect of stroke recurrence between hsCRP and functional disability. This study would provide important information on the mechanism of functional disability after stroke and provide valuable insight on the role of adding anti-inflammatory therapy to antithrombotic, antihypertensive, and lipid-lowering strategies for functional improvement after stroke.

The detailed mechanism of reduced functional outcome after a stroke is not fully understood yet, and it is related to a complex cycle of interconnected molecular and cellular mechanisms.³¹ However, evidence clearly supports that inflammation contributes to cell death, brain injury, and blood-brain barrier disruption, all of which would increase the cerebral infarct size and cause further functional damage.^{9–12} In addition, inflammation also plays a critical role in the pathogenesis and progression of atherosclerosis, plaque rupture, platelet aggregation, and intravascular thrombosis, all of which increase the risk of stroke and then result in functional disability.¹³ Some discussions mentioned that inflammation in the chronic phase after stroke may promote tissue repair and functional recovery.³² However, blood was sampled within 24 h of admission (median 55 h after the event) in our analysis, and CRP or hsCRP is an acute-phase protein of inflammatory processes.^{33,34} Therefore, the current analysis mainly reflected the effect acute phase of inflammatory and would add evidence for the important role of anti-inflammatory therapy at the acute stage of ischaemic stroke.

As our analysis revealed that less than 20% of functional outcome variance could be explained by stroke recurrence, which means that over 80% of functional

damage results from the pathway between hsCRP and disability without the mediation of stroke recurrence. Therefore, the typical secondary prevention strategies for preventing stroke recurrence are far from sufficient to improve the prognosis of stroke patients, and anti-inflammatory therapy should be given more attention from the perspectives of both acute-phase and late phase mechanisms. New therapies targeting poststroke inflammation, i.e., cytokine inhibition, should be pursued experimentally and clinically in well-selected subjects at high risk, either alone or in combination with thrombolysis and/or thrombectomy.³⁵ Previous studies aiming to assess the effects of targeted cytokine antagonist therapies for atherothrombosis showed that targeting certain inflammatory cytokines holds promise.^{36,37}

This study has several limitations. First, 3781 (33.2%) of 11 384 study participants were excluded from this analysis because of missing data on hsCRP or missing data on 90-day follow-up mRS, which may introduce selection bias. Since methods of directly testing missing at random is not available yet, we are not confident to state that the excluded data can be considered to be missing at random.³⁸ Nevertheless, we believe that the selection bias would be minimized in this study as the baseline characteristics between included and excluded patients were largely comparable. Second, although blood samples were collected within 24 h of admission, patients were recruited up to 7 days after onset, which may introduce heterogeneity. However, the median time for blood sample collection was 55 h after symptom onset, which would reduce the heterogeneity. In addition, results of mediation analysis stratified by admission time showed comparable mediation effects, which indicated that the mediation effects would not be affected by admission time or by the time of blood sample collection. Third, during hospitalization, pulmonary

infection occurred among 381 (5.0%), and urinary system infection occurred among 110 (1.5%) of the included patients. However, we could not precisely judge whether these infections occurred before or after blood samples were taken. Forth, blood laboratory data usually have a skewed distribution, which may bias the estimation of mediating effects. However, sensitivity analysis on the log-scale, and per SD of the log-scale of hsCRP revealed consistent and robust results, which made our conclusion more reliable. Fifth, although we carefully controlled for potential covariates when assessing the mediating effect, other unmeasured confounders may still exist. Sixth, we did not perform mediation analyses considering hospital clustering because no reliable methods developed under the counterfactual framework are available yet. In addition, the intra-class correlation coefficients for hsCrp, stroke recurrence, and functional disability in this study were as low as 0.04, 0.03, and 0.01, respectively, which means the clustering effect of hospitals is small and would be minimally biased. Seventh, although the mRS is a well-validated and accepted scale, it remains a subjective patient-derived measure. The extent to which functional change can be captured by mRS scores may need further investigation. Finally, only 247 (3.2%) were ethnic minorities; acute ischaemic stroke patients with prior functional disability or patients who received reperfusion treatment were excluded from this analysis. Therefore, generalizing our conclusions to these populations should be with caution.

In conclusion, stroke recurrence mediates less than 20% of the association between hsCRP and functional outcomes at 90 days in patients with ischaemic stroke, suggesting that novel anti-inflammatory therapy should be given more attention to improving functional outcomes.

Contributors

HQG, ZXL, and YJW conceptualised and designed the study. JXL, JJ, XM, YJ, HQG, ZXL collected the data. HQG drafted the manuscript, analysed and interpreted the data. HQG and KXY analysed the data. HQG, KXY, and ZXL verified the underlying data. XQZ, YLW, LPL, ZXL, HL, and YJW interpreted the data and revised the manuscript. All authors commented upon and approved the final manuscript.

Data sharing statement

The data used for this analysis can be made available upon reasonable request to the corresponding authors.

Declaration of interests

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.ebiom.2022.104054](https://doi.org/10.1016/j.ebiom.2022.104054).

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