The Contribution of Inflammation to Stroke Recurrence Attenuates at Low LDL-C Levels

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Aims: Residual inflammation risk refers to inflammation that still increases the risk of cardiovascular disease after the level of low-density lipoprotein cholesterol (LDL-C) reached the target (<70 mg/dL). However, whether inflammation is still an important issue even if very low LDL-C levels have been achieved remains unclear. This study aimed to investigate the contribution of inflammation to stroke recurrence on different LDL-C levels following ischemic stroke (IS) or transient ischemic attack (TIA).

Methods: A total of 10499 IS/TIA patients whose LDL-C and high-sensitivity C-reactive protein (hsCRP) were measured were selected from the Third China National Stroke Registry. The cutoff values were set to 25, 35, 45, 55, 70, and 100 mg/dL for LDL-C, whereas the threshold values of hsCRP and interleukin-6 (IL-6) were 2 mg/L and 1.65 ng/L, respectively. Based on each group of LDL-C, Cox regressions were conducted to investigate the associations between inflammation and recurrent stroke within 1 year.

Results: The associations between baseline hsCRP levels and stroke recurrence were non-significant in groups with LDL-C <55 mg/dL (P > 0.05). After stratification by baseline LDL-C of 55 mg/dL, hsCRP ≥ 2 mg/L (10.9% versus 7.5%, P < 0.0001) and IL-6 ≥ 1.65 ng/L (9.8% versus 7.4%, P = 0.0002) were found to be related to a high incidence of recurrent IS among patients with LDL-C ≥ 55 mg/dL; however, no associations were observed among patients with LDL-C <55 mg/dL. Compared with low inflammation (both hsCRP <2 mg/L and IL-6 <1.65 ng/L), high inflammation (both hsCRP ≥ 2 mg/L and IL-6 ≥ 1.65 ng/L) was significantly associated with stroke recurrence when LDL-C ≥ 55 mg/dL (adjusted HR 1.38, 95% CI 1.10–1.74), whereas this association was not observed when LDL-C <55 mg/dL (adjusted HR, 0.72; 95% CI, 0.41–1.25).

Conclusion: For IS/TIA patients, the contribution of inflammation to stroke recurrence seems to be attenuated at a low level of LDL-C.

Key words: Stroke recurrence, Inflammation, Low-density lipoprotein cholesterol, High-sensitivity C-reactive protein, Interleukin-6

Introduction

Elevated low-density lipoprotein cholesterol (LDL-C) is a well-established risk factor for atherosclerosis1). Data from SPARCL (Stroke Prevention by Aggressive Reduction in Cholesterol
Levels\textsuperscript{2, 3} and the TST (Treat Stroke to Target) trial\textsuperscript{4} have demonstrated that an LDL-C concentration that is intensively reduced to the target level (\(<70\text{ mg/L}\)) could effectively reduce the risk of stroke recurrence.

However, studies demonstrated that even though LDL-C was controlled to achieve the target level after contemporary lipid-lowering therapy, residual risks still remain\textsuperscript{5-7}. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) established the cornerstone status of residual inflammation risk in cardiovascular diseases, which indicated that anti-inflammatory therapy with canakinumab reduced the levels of high-sensitivity C-reactive protein (hsCRP) and then effectively reduced recurrent cardiovascular events by 27%. This suggests that residual inflammation is a modifiable risk factor and proposes a potential treatment target for patients with cardiovascular disease\textsuperscript{5}.

Lipid metabolism plays a role in the modulation of inflammation and immunity\textsuperscript{8}. Furthermore, previous studies have demonstrated that elevated hsCRP levels are always positively correlated with LDL-C levels\textsuperscript{9, 10}. Studies have confirmed that lipoprotein metabolism disorder could induce inflammatory and immune responses in the formation and progression of atherosclerosis\textsuperscript{11}. The question is whether the inflammatory level will decrease and the contribution of inflammation to cardiovascular event will be attenuated if the LDL-C concentration is reduced to an extremely low level. Therefore, we hypothesize that the influence of inflammation on the risk of cardiovascular disease decreases with the decrease in the LDL-C levels. This study aimed to investigate this issue among patients with ischemic stroke (IS) or transient ischemic attack (TIA) in Chinese population based on data from the Third China National Stroke Registry (CNSR III).

We present the following article in accordance with the STROBE reporting checklist.

**Materials and Methods**

**Study Design and Population**

The CNSR III is a large-scale, nationwide, hospital-based, prospective registry for IS and TIA. A total of 201 hospitals covering 22 provinces and four municipalities in China participated in this registry. In total, 15166 patients with IS and TIA were recruited between August 2015 and March 2018. Of these hospitals, 171 with 11261 patients participated in the biomarker substudy; the blood samples of these patients were sent to the central laboratory. The study’s detailed design and rationale as well as the baseline patient characteristics were recently described\textsuperscript{12}. The protocol and data collection methods of the CNSR III were approved by the ethics committee of all participating hospitals. Furthermore, written informed consent was obtained from the participants or their legally authorized representatives.

We analyzed the data obtained from the CNSR III. Patients with IS/TIA from the subcenters of the biomarker study were included in our analysis. IS was diagnosed according to the WHO criteria\textsuperscript{13} with confirmation by brain magnetic resonance (MR) imaging or computerized tomography (CT). Patients with missing data for baseline LDL-C and hsCRP levels were excluded from the analysis.

**Baseline Data**

The baseline clinical data of patients enrolled in the CNSR III was collected by trained research coordinators at each institute, and the study investigators and research coordinators were trained. Baseline information was collected in detail, including age, sex, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared (kg/m\textsuperscript{2})), previously used lipid-lowering agents (including statins, fibrates, acid chelating resins, nicotinic acid and its derivatives, and Zhbituo), medical history (prior stroke, coronary heart disease, hypertension, diabetes mellitus, hyperlipidemia, and atrial fibrillation), etiology classification of ischemic stroke (performed according to the T rial of Org 10172 in the Acute Stroke T reatment (TOAST) criteria\textsuperscript{14} ), antiplatelet (single or dual antiplatelet therapy, including aspirin, clopidogrel, dipyridamole, cilostazol) and lipid-lowering therapy at discharge, blood pressure (BP), and fasting plasma glucose (FPG) upon admission.

**Blood Sample**

Blood samples were collected within 24 h of admission and at 3-month follow-up from 171 subcenters that participated in the biomarker substudy. Fasting blood samples were collected in serum-separating tubes and EDTA anticoagulant blood collection tubes. Finally, the blood samples were transported through cold chain to the central laboratory. The lipid levels, including the LDL-C and high-density lipid cholesterol (HDL-C), were measured by enzymatic method on Cobas 8000 analyzer c702 module (Roche Diagnostics, Mannheim, Germany). The hsCRP concentrations were measured on a Roche Cobas C701 analyzer. The level of interleukin-6 (IL-6) was measured using enzyme-linked immunosorbent assay kits (catalog number: PHS600C, Inc., Minneapolis, MN, USA).
Primary Outcome

The patients were interviewed face-to-face at 3-month follow-up and contacted over the telephone by the trained research coordinators at 6-month and 1-year follow-up. Information on cardiovascular/cerebrovascular events was queried at each follow-up. Confirmation of vascular events was sought from the treating hospital, and suspected recurrent cerebrovascular events without hospitalization were judged by an independent endpoint judgment committee. The primary outcome was a new ischemic stroke within 1 year, which was defined as an aggravated primary neurological deficit (an increase in the NIHSS score by 4 points or above), a new neurological deficit lasting more than 24 h, and imaging-confirmed (CT or MR) new infarction lesions or expansion of the original infarction or re-hospitalization with a diagnosis of ischemic stroke.

Statistical Analysis

Categorical variables were expressed as percentages and continuous variables as medians with interquartile ranges (IQR). The baseline characteristics between the different LDL-C level groups were compared using chi-squared statistics for the categorical variables and one-way analysis of variance (ANOVA) or Kruskal–Wallis test for the continuous variables.

Two hsCRP categories were constructed with a cutoff value of 2 mg/L. The LDL-C cutoff values were set to 25, 35, 45, 55, 70, and 100 mg/dL to divide patients into seven groups with LDL-C levels of <25, 25–35, 35–45, 45–55, 55–70, 70–100, and ≥100 mg/dL, respectively. Individuals were categorized using these hsCRP and LDL-C cutoff values. Associations between high inflammation level and risk of stroke recurrence in each group of LDL-C were investigated using Cox proportional-hazards models.

Furthermore, the LDL-C cutoff value of 55 mg/dL (derived from the above analysis, indicating that there are significant associations between inflammation risk and stroke recurrence in groups with LDL-C levels of 55–70, 70–100, and ≥100 mg/dL but no associations in groups with LDL-C levels of 45–55, 35–45, 25–35, and <25 mg/dL) was utilized to categorize patients into two groups (≤55 and ≥55 mg/dL) in order to investigate the association between inflammation (assessed by hsCRP and IL-6 (the IL-6 cutoff value of 1.65 ng/L was used based on previous study)) and the risk of stroke recurrence. Kaplan–Meier survival curves were used to depict the associations of hsCRP and IL-6 with stroke recurrence, respectively, which were analyzed using the logrank univariate test. Then, combining the indices of hsCRP and IL-6, we used the Cox proportional-hazards models to analyze the associations between the different inflammation status (four strata: hsCRP <2 mg/L and IL-6 <1.65 ng/L, hsCRP <2 mg/L and IL-6 ≥1.65 ng/L, hsCRP ≥2 mg/L and IL-6 <1.65 ng/L, and hsCRP ≥2 mg/L and IL-6 ≥1.65 ng/L) and the risk of stroke recurrence; the hazard ratios (HR) with their 95% confidence intervals (CI) were calculated in two models (unadjusted model and adjusted model: adjusted for age, sex, and other potential confounding factors). In addition, using the hsCRP and LDL-C levels at baseline and 3-month, we evaluated the associations between persistent high inflammation and recurrent stroke based on persistent and non-persistent high LDL-C level. Persistent high inflammation was defined as hsCRP level ≥2 mg/L both at baseline and 3-month and persistent high LDL-C level as LDL-C level ≥55 mg/dL both at baseline and 3-month. Cox proportional-hazards models were used.

In the subgroup analyses, the LDL-C and hsCRP cutoff values of 55 mg/dL and 2 mg/L, respectively, were used. The associations between inflammation risk and recurrent stroke based on the different LDL-C groups were investigated among patients with large artery atherosclerosis (LAA) subtype, patients with diabetes mellitus, patients without cerebrovascular disease, and patients not using lipid-lowering agents. A two-sided P-value of <0.05 was considered to indicate statistical significance. The SAS software version 9.4 (SAS Institute, Inc., Cary, NC) was employed for all statistical analyses.

Results

Baseline Characteristics

A total of 15166 IS/TIA patients were enrolled in the CNSR III. Of them, 11261 patients from 171 hospitals participated in the biomarker substudy, and the blood samples of these patients were sent to the central laboratory. After excluding patients with missing data of the baseline LDL-C and hsCRP, 10499 patients were finally included in the analysis (Supplemental Fig.1). The patients included and those excluded in this analysis were well balanced (Supplemental Table 1).

There were 102 (1.0%), 178 (1.7%), 395 (3.8%), 762 (7.2%), 1540 (14.7%), 3481 (33.1%), and 4041 (38.5%) patients with LDL-C levels of <25, 25–35, 35–45, 45–55, 55–70, 70–100, and ≥100 mg/dL, respectively. The baseline clinical characteristics of patients according to the LDL-C levels are presented in Supplemental Table 2. At
Inflammation, LDL-C, and Ischemic Stroke

Table 1. Baseline Clinical Characteristics according to Baseline hsCRP Concentrations

| Variable                                      | hsCRP < 2 mg/L, n=5598 | hsCRP ≥ 2 mg/L, n=4901 | P value |
|-----------------------------------------------|------------------------|-------------------------|---------|
| Age, years, median (IQR)                      | 61 (53.68)             | 64 (56.72)              | <0.0001 |
| Male, n (%)                                   | 3900 (69.7)            | 3275 (66.8)             | 0.002   |
| BMI, kg/m², median (IQR)                      | 24.3 (22.5,26.3)       | 24.6 (22.7,26.8)        | <0.0001 |
| Previous use of lipid-lowering drugs, n (%)   | 667 (11.9)             | 558 (11.4)              | 0.40    |
| Medical history, n (%)                        |                        |                         |         |
| Hypertension                                  | 3423 (61.2)            | 3183 (65.0)             | <0.0001 |
| Diabetes mellitus                             | 1241 (22.2)            | 1270 (25.9)             | <0.0001 |
| Dyslipidemia                                  | 439 (7.8)              | 425 (8.7)               | 0.12    |
| Cardiocerebrovascular diseases                | 1554 (27.8)            | 1590 (32.4)             | <0.0001 |
| Index event, n (%)                            |                        |                         | <0.0001 |
| IS                                            | 5141 (91.8)            | 4643 (94.7)             |         |
| TIA                                           | 457 (8.2)              | 258 (5.3)               |         |
| TOAST subtype, n (%)                          |                        |                         | <0.0001 |
| LAA                                           | 1228 (21.9)            | 1427 (29.1)             |         |
| Non-LAA                                       | 4370 (78.1)            | 3474 (70.9)             |         |
| Medication use, n (%)                         |                        |                         |         |
| Lipid-lowering drugs                          | 5230 (93.5)            | 4479 (91.8)             | 0.0005  |
| Antiplatelet agents                           | 5221 (93.4)            | 4391 (90.0)             | <0.0001 |
| BP at admission, mmHg, median (IQR)           | 148 (135,163)          | 150 (136,165)           | 0.001   |
| FPG, mmol/L, median (IQR)                     | 5.40 (4.84,6.58)       | 5.72 (4.98,7.28)        | <0.0001 |
| Baseline lipid levels, mmol/L, median (IQR)   |                        |                         |         |
| LDL-C                                         | 2.28 (1.69,2.93)       | 2.37 (1.77,3.06)        | <0.0001 |
| HDL-C                                         | 1.09 (0.92,1.31)       | 1.05 (0.88,1.25)        | <0.0001 |
| TG                                            | 1.38 (1.03,1.89)       | 1.36 (1.03,1.86)        | 0.55    |
| hsCRP, mg/L, median (IQR)                     | 0.86 (0.55, 1.25)      | 5.10 (3.06, 11.21)      | <0.0001 |
| IL-6, ng/L, median (IQR)                      | 1.90 (1.28, 2.90)      | 4.28 (2.47, 8.24)       | <0.0001 |

Abbreviations: IQR=interquartile range; BMI=body mass index; LAA=large artery atherosclerosis; non-LAA=non-large artery atherosclerosis; BP=blood pressure; FPG=fasting plasma glucose; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglycerides; hsCRP=high-sensitivity C-reactive protein; IL-6=interleukin 6.

BMI is the weight in kilograms divided by the square of the height in meters. TOAST denotes stroke etiology classification criteria according to the Trial of Org 10172 in Acute Stroke Treatment.

Baseline, 4901 (46.7%) patients had an hsCRP level equal to or more than 2 mg/L. The baseline characteristics mostly differed according to the hsCRP levels (Table 1).

**Associations between Inflammation Risk and Stroke Recurrence According to the LDL-C Level**

At 1 year, a total of 936 (8.9%) patients developed a new ischemic stroke. Patients who were older; female; with diabetes mellitus and cardiocerebrovascular diseases; diagnosed with IS and an LAA subtype; did not receive lipid-lowering and antiplatelet therapy; and had higher levels of BP, FPG, LDL-C, hsCRP, and IL-6 tended to have a higher risk of recurrent stroke (Supplemental Table 3).

From the highest LDL-C (≥ 100 mg/dL) to the lowest LDL-C (<25 mg/dL) group, the rate differences of recurrent stroke between the high and low hsCRP levels were 3.6%, 3.1%, 3.3%, 1.8%, −1.4%, 1.5%, and 0.7% (Fig. 1). On each group of LDL-C level, the HRs of stroke recurrence among patients with hsCRP ≥ 2 mg/L were 1.50 (1.23–1.83), 1.48 (1.18–1.86), 1.50 (1.07–2.12), 1.33 (0.78–2.26), 0.86 (0.42–1.73), 1.22 (0.44–3.37), and 1.13 (0.27–4.73) compared with those with hsCRP < 2 mg/L, respectively. While the LDL-C concentration decreased to 55 mg/dL and below, we failed to find significant association between residual inflammation risk and stroke recurrence (Fig. 2).

Subsequently, we stratified the patients by the LDL-C cutoff value of 55 mg/dL for further analysis. The results indicated that hsCRP ≥ 2 mg/L (10.9% versus 7.5%, P<0.0001), and IL-6 ≥ 1.65 ng/L (9.8% versus 7.4%, P=0.0002) were related to a high incidence of recurrent stroke among patients with LDL-C ≥ 55 mg/dL; however, no association was observed among those with LDL-C < 55 mg/dL (Fig. 3). In the group of LDL-C ≥ 55 mg/dL, the risk
observed in the non-persistent high LDL-C group (9.8% versus 7.5%, adjusted HR 1.20, 95% CI 0.81–1.79) (Table 2).

Subgroup Analysis

After classifying patients according to the TOAST criteria, LAA subtype accounted for about 25.3%; of these, a total of 319 (12.0%) patients developed a new ischemic stroke within 1 year. Inflammation was significantly associated with recurrent stroke in the LDL-C ≥55 mg/dL group (14.0% versus 10.2%, adjusted HR 1.47, 95% CI 1.11–1.95), whereas this association was not observed in the LDL-C <55 mg/dL group (10.8% versus 10.1%, adjusted HR 0.88, 95% CI 0.38–2.02) (Fig. 4).

Furthermore, we investigated these associations specifically in patients with diabetes mellitus, without cardiocerebrovascular disease, and without previous use of lipid-lowering agents. As presented in Supplemental Fig. 2, similar trends were observed in these three subgroup patients.
This study demonstrated that, among IS/TIA patients, the contribution of inflammation to recurrent stroke gradually decreases from the highest LDL-C to the lowest LDL-C stratum. Inflammation was found to be associated with a high risk of recurrent stroke when the LDL-C level was $\geq 55$ mg/dL; however, this association was not observed when the LDL-C level decreased to 55 mg/dL and below.

Recently, two studies using data from the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) and SPIRE (Studies of PCSK9 Inhibition and Reduction of Vascular Events) trials have addressed the interesting question of whether inflammation is still an important issue even after very low LDL-C levels have been achieved. They found that, even at extremely low levels of LDL-C (as low as $<20$ mg/dL), inflammatory processes still play a role in cardiovascular disease\textsuperscript{17, 18}. However, in the analysis based on the FOURIER trial\textsuperscript{17}, we also found that, as the LDL-C level decreases, the rate difference of cardiovascular events between high and low hsCRP level also decreases. In the FOURIER trial, the rate difference dropped from 5.9% (18.2%–12.3%) to 4.1% (13.1%–9.0%), from the highest LDL-C ($\geq 100$ mg/dL) to the lowest LDL-C ($\leq 25$ mg/dL) group. To some extent, this trend is consistent with our results, indicating that the rate difference of recurrent stroke between high and low hsCRP decreases from 3.6% to 0.7% from the highest LDL-C ($\geq 100$ mg/dL) to the lowest LDL-C ($<20$ mg/dL) group. Moreover, our results indicated that the rate difference dropped more significantly. However, we did not find any association between the hsCRP level and recurrent stroke among patients with LDL-C levels $<55$ mg/dL. We believe that this non-significant association between hsCRP and recurrent stroke is related to the insufficient sample size of the low-LDL-C group in our study.
The CANTOS study did not strictly limit the LDL-C levels in its inclusion criteria. Therefore, future randomized controlled trials are needed to confirm whether canakinumab can further reduce the risk of cardiovascular and cerebrovascular events at extremely low LDL-C levels, such as 55 mg/dL (1.0 mmol/L), as suggested by the ESC 2019 guideline for very high-risk patients.\textsuperscript{19} Moreover, previous studies have confirmed that lipid-lowering agents have anti-inflammatory effects,\textsuperscript{11, 20-22} but whether these anti-inflammatory effects are either independent of a decrease in low-density lipoprotein cholesterol remains uninvestigated. Hence, we do not think that the effect of residual inflammation risk will disappear at very low LDL-C levels, but it will be attenuated.

With the advent of the PCSK9 inhibitors, the LDL-C level of most patients may be controlled to reach an extremely low level. Based on our findings, the questions that need to be considered are whether the contribution of inflammation to cardiovascular disease risk at extremely low LDL-C levels will decrease and whether it is necessary to use PCSK9 inhibitors in combination with canakinumab, considering the expensive price of the two antibodies. 

### Table 2. Associations of Persistent High Inflammation with Recurrent Ischemic Stroke Based on Different Status of LDL-C

|                          | n (%)    | Unadjusted model | Adjusted model* |
|--------------------------|----------|------------------|-----------------|
|                          |          | HR   | 95% CI     | P    | HR   | 95% CI     | P    |
| Non-persistent high LDL-C level\textsuperscript{1} |          |      |            |      |      |            |      |
| Non-persistent high inflammation\textsuperscript{2} | 112/1498 (7.5) | Ref. |            |      | Ref. |            |      |
| Persistent high inflammation\textsuperscript{2}     | 33/336 (9.8)  | 1.33  | 0.90–1.96 | 0.15 | 1.20 | 0.81–1.79 | 0.35 |
| Persistent high LDL-C level\textsuperscript{3}     |          |      |            |      |      |            |      |
| Non-persistent high inflammation | 221/2898 (7.3) | Ref. |            |      | Ref. |            |      |
| Persistent high inflammation     | 92/874 (10.5) | 1.41  | 1.11–1.80 | 0.005| 1.29 | 1.01–1.64 | 0.04 |

\textsuperscript{1}Persistent high LDL-C level refers to LDL-C ≥ 55 mg/dL both at baseline and 3-month, all other statuses of LDL-C were defined as non-persistent high LDL-C level. \textsuperscript{2}Persistent high inflammation was defined as hsCRP level ≥ 2 mg/L both at baseline and 3-month, other status of hsCRP were defined as non-persistent high inflammation. \textsuperscript{3}Adjusted for age, sex, medical history of diabetes mellitus, cardiocerebrovascular diseases, TOAST classification.

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|                          | n (%)    | Unadjusted model | Adjusted model* |
|--------------------------|----------|------------------|-----------------|
|                          |          | HR   | 95% CI     | P    | HR   | 95% CI     | P    |
| Non-persistent high LDL-C level\textsuperscript{1} |          |      |            |      |      |            |      |
| Non-persistent high inflammation\textsuperscript{2} | 112/1498 (7.5) | Ref. |            |      | Ref. |            |      |
| Persistent high inflammation\textsuperscript{2}     | 33/336 (9.8)  | 1.33  | 0.90–1.96 | 0.15 | 1.20 | 0.81–1.79 | 0.35 |
| Persistent high LDL-C level\textsuperscript{3}     |          |      |            |      |      |            |      |
| Non-persistent high inflammation | 221/2898 (7.3) | Ref. |            |      | Ref. |            |      |
| Persistent high inflammation     | 92/874 (10.5) | 1.41  | 1.11–1.80 | 0.005| 1.29 | 1.01–1.64 | 0.04 |

\textsuperscript{1}Persistent high LDL-C level refers to LDL-C ≥ 55 mg/dL both at baseline and 3-month, all other statuses of LDL-C were defined as non-persistent high LDL-C level. \textsuperscript{2}Persistent high inflammation was defined as hsCRP level ≥ 2 mg/L both at baseline and 3-month, other status of hsCRP were defined as non-persistent high inflammation. \textsuperscript{3}Adjusted for age, sex, medical history of diabetes mellitus, cardiocerebrovascular diseases, TOAST classification.

**Fig. 4.** Hazard ratios of stroke recurrence according to hsCRP and IL-6. A, in the group of LDL-C < 55 mg/dL; B, in the group of LDL-C ≥ 55 mg/dL.

*Adjusted for age, sex, BP at admission, FPG at baseline, medical history of diabetes mellitus, cardiocerebrovascular diseases, TOAST classification, and use of lipid-lowering drugs and antiplatelet agents.

Hence, we do not think that the effect of residual inflammation risk will disappear at very low LDL-C levels, but it will be attenuated.
unclear. Further studies are needed to compare lipid-lowering and anti-inflammatory agents and a combination of both therapies to help develop treatment strategies.

Our data indicated that inflammation risk decreases with the decrease in the LDL-C levels, which can be explained by the correlation between lipid metabolism and inflammation and immune response. It is well recognized that elevated LDL level is an important cause of inflammation in atherosclerosis, indicating that inflammation level in atherosclerosis may be positively related to LDL-C levels. In the process of atherosclerosis, increased LDL-C levels stimulate the release of inflammatory factors or induce an inflammatory response through a variety of mechanisms. Oxidized LDL-C was involved in the production of co-stimulatory molecules (CD80, CD86, and CD40), proinflammatory eicosanoids, proinflammatory cytokines, etc. Monocytes were recruited to areas with LDL-induced endothelial damage, and LDL contributed to cell morphology and viability at the early stages of monocyte to macrophage differentiation. In addition, dietary lipid modifications were also found to be associated with immune response. Low-fat diet was confirmed to contribute to the reduction of hsCRP levels.

Strengths and Limitations

This study not only used hsCRP but also selected its upstream IL-6 as an inflammation marker, which enhanced the consistency of our results. However, our study has several limitations. First, due to the limited sample size of very low LDL-C group, our results should be interpreted with caution. Second, our primary analysis was based on baseline LDL-C and hsCRP levels, which led to overlook on the effects of medicine therapy following IS/TIA. Although we further evaluated the dynamic changes of inflammation and LDL-C levels using the hsCRP and LDL-C concentrations at baseline and 3 months, the results may still be biased due to the lack of data on LDL-C and inflammation levels during the occurrence of recurrent events and changes of treatment strategies after stroke recurrence. Third, this study was conducted in a Chinese patient population; thus, our findings may not be generalizable to other populations.

Conclusion

In summary, for IS/TIA patients, we found that, the contribution of inflammation to stroke recurrence seems likely to attenuate at a low LDL-C level. Future randomized controlled trials are needed to verify whether anti-inflammatory therapy could further reduce the risk of cardiovascular disease at extremely low LDL-C levels.

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**Supplemental Table 1.** Baseline Clinical Characteristics of Study Patients and Rest Patients in the CNSR III

| Characteristic                                      | Include, n = 10499 | Exclude, n = 762 | P value |
|-----------------------------------------------------|--------------------|------------------|---------|
| Age, years, median (IQR)                           | 63 (54,70)         | 62 (54,70)       | 0.91    |
| Male, n (%)                                        | 7175 (68.3)        | 546 (71.6)       | 0.06    |
| BMI, kg/m², median (IQR)                           | 24.5 (22.6,26.6)   | 24.6 (22.5,26.5) | 0.85    |
| Previous lipid-lowering agents use, n (%)          | 1225 (11.7)        | 91 (11.9)        | 0.82    |
| Medical history, n (%)                             |                    |                  |         |
| Hypertension                                        | 6606 (62.9)        | 463 (60.8)       | 0.23    |
| Diabetes mellitus                                   | 2511 (23.9)        | 178 (23.4)       | 0.73    |
| Dyslipidemia                                        | 864 (8.2)          | 70 (9.2)         | 0.36    |
| Cardiocerebrovascular diseases                      | 3144 (30.0)        | 231 (30.3)       | 0.83    |
| Index event, n (%)                                  | 9784 (93.2)        | 707 (92.8)       | 0.67    |
| IS                                                  | 715 (6.8)          | 55 (7.2)         |         |
| TIA                                                | 9784 (93.2)        | 707 (92.8)       | 0.67    |
| TOAST classification, n (%)                         | 2655 (25.3)        | 178 (23.4)       | 0.24    |
| LAA                                                | 7844 (74.7)        | 584 (76.6)       |         |
| Non-LAA                                            |                    |                  |         |
| Medication use, n (%)                               | 9709 (92.7)        | 698 (91.7)       | 0.45    |
| Lipid-lowering drugs                                | 9612 (91.8)        | 689 (90.8)       | 0.33    |
| Antplatelet agents                                  | 149 (135,164)      | 149 (135,163)    | 0.58    |
| BP at admission, mmHg, median (IQR)                | 5.53 (4.90,6.89)   | 5.60 (4.92,6.94) | 0.42    |

**Abbreviations:** IQR = interquartile range; BMI = body mass index; LAA = large artery atherosclerosis; non-LAA = non-large artery atherosclerosis; BP = blood pressure; FPG = fasting plasma glucose. BMI is the weight in kilograms divided by the square of the height in meters. TOAST denotes stroke etiology classification criteria according to the Trial of Org 10172 in Acute Stroke Treatment.
### Supplemental Table 2. Baseline Clinical Characteristics by Baseline LDL-C levels

| Characteristic | <25 mg/dL, n=102 | 25-35 mg/dL, n=395 | 35-45 mg/dL, n=762 | 45-55 mg/dL, n=1540 | 55-70 mg/dL, n=3481 | 70-100 mg/dL, n=2473 | ≥ 100 mg/dL, n=2523 | P value |
|---------------|-------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------|
| Age, years, median (IQR) | 58 (49, 65) | 60 (52, 69) | 61 (53, 69) | 61 (53, 69) | 63 (54, 70) | 62 (54, 70) | 63 (55, 71) | <0.0001 |
| Male, n (%) | 76 (74.5) | 128 (71.9) | 295 (74.7) | 552 (72.4) | 1128 (73.2) | 2473 (71.0) | 2523 (62.4) | <0.0001 |
| BMI, kg/m², median (IQR) | 24.8 (23.4, 26.6) | 25.0 (22.9, 27.5) | 24.5 (22.5, 26.3) | 24.2 (22.3, 26.1) | 24.5 (22.6, 26.7) | 24.5 (22.6, 26.4) | 24.5 (22.6, 26.7) | 0.004 |
| Previous lipid-lowering agents use, n (%) | 21 (20.6) | 41 (23.0) | 73 (18.5) | 134 (17.6) | 210 (13.6) | 379 (10.9) | 367 (9.1) | <0.0001 |
| Medical history, n (%) | 62 (60.8) | 119 (66.9) | 267 (67.6) | 469 (61.6) | 981 (63.7) | 2192 (63.0) | 2516 (62.3) | 0.33 |
| Hypertension | 30 (29.4) | 54 (30.3) | 120 (30.4) | 187 (24.5) | 368 (23.9) | 786 (22.6) | 977 (23.9) | 0.005 |
| Diabetes mellitus | 12 (11.8) | 15 (8.4) | 31 (7.9) | 61 (8.0) | 112 (7.3) | 287 (8.2) | 346 (8.6) | 0.64 |
| Dyslipidemia | 27 (26.5) | 69 (38.8) | 145 (36.7) | 252 (33.1) | 533 (34.6) | 975 (28.0) | 1143 (28.3) | <0.0001 |
| Cardiocerebrovascular diseases | 91 (89.2) | 165 (92.7) | 367 (92.9) | 721 (94.6) | 1442 (93.6) | 3253 (93.5) | 3745 (92.7) | 0.26 |
| Index event, n (%) | 11 (10.8) | 13 (7.3) | 28 (7.1) | 41 (5.4) | 98 (6.4) | 228 (6.5) | 296 (7.3) | 0.20 |
| TOAST classification, n (%) | 22 (21.6) | 38 (21.3) | 86 (21.7) | 170 (22.3) | 361 (23.4) | 892 (25.6) | 1086 (26.9) | 0.71 |
| LAA | 80 (78.4) | 140 (78.7) | 309 (78.3) | 592 (77.7) | 1179 (76.6) | 2589 (74.4) | 2955 (73.1) | 0.85 |
| Non-LAA | 92 (90.2) | 167 (93.8) | 369 (93.7) | 694 (91.6) | 1433 (93.1) | 3216 (92.6) | 3738 (92.8) | 0.71 |
| Medication use | 95 (93.1) | 168 (94.4) | 305 (92.6) | 594 (91.6) | 1415 (91.94) | 3178 (91.5) | 3697 (91.8) | 0.85 |
| Lipid-lowering drugs | 145 (133, 160) | 144 (134, 157) | 145 (132, 162) | 145 (133, 161) | 147 (133, 161) | 149 (135, 164) | 150 (136, 167) | <0.0001 |
| Antiplatelet agents | 5.42 (4.88, 7.11) | 5.39 (4.78, 6.84) | 5.56 (4.80, 7.07) | 5.43 (4.82, 6.74) | 5.42 (4.88, 6.78) | 5.46 (4.84, 6.62) | 5.65 (4.97, 7.23) | <0.0001 |
| BP at admission, mmHg, median (IQR) | 0.50 (0.37, 0.57) | 0.79 (0.71, 0.85) | 1.05 (0.98, 1.11) | 1.31 (1.25, 1.37) | 1.62 (1.53, 1.72) | 2.19 (2.00, 2.38) | 3.19 (2.86, 3.74) | <0.0001 |
| FPG, mmol/L, median (IQR) | 0.86 (0.70, 1.00) | 0.91 (0.77, 1.15) | 0.97 (0.80, 1.15) | 0.99 (0.82, 1.20) | 1.01 (0.85, 1.23) | 1.05 (0.90, 1.24) | 1.15 (0.98, 1.37) | <0.0001 |
| HDL-C | 2.09 (0.98, 4.18) | 1.41 (0.89, 2.68) | 1.23 (0.92, 1.93) | 1.25 (0.92, 1.75) | 1.26 (0.96, 1.81) | 1.32 (1.00, 1.76) | 1.47 (1.13, 1.96) | 0.0001 |
| hsCRP at baseline, mg/L, median (IQR) | 1.17 (0.56, 2.87) | 1.62 (0.76, 3.92) | 1.67 (0.72, 5.24) | 1.47 (0.77, 4.08) | 1.65 (0.78, 4.52) | 1.77 (0.82, 4.60) | 1.94 (0.89, 4.93) | 0.0001 |
| IL-6, ng/L, median (IQR) | 3.21 (1.76, 5.58) | 2.94 (1.70, 7.68) | 2.73 (1.65, 5.12) | 2.59 (1.56, 4.80) | 2.77 (1.71, 5.45) | 2.66 (1.58, 5.11) | 2.53 (1.54, 4.69) | 0.003 |

Abbreviations: IQR=interquartile range; LAA=large artery atherosclerosis; non-LAA=non-large artery atherosclerosis; BP=blood pressure; FPG=fasting plasm glucose; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglycerides; hsCRP=high-sensitivity c-reactive protein; IL-6=interleukin 6.

BMI is the weight in kilograms divided by the square of the height in meters. TOAST denotes stroke etiology classification criteria according to the Trial of Org 10172 in Acute Stroke Treatment.
### Supplemental Table 3. Baseline Clinical Characteristics of Patients Had Ischemic Stroke Recurrence and Those Had not

| Variable                               | Stroke recurrence (No), n=9563 | Stroke recurrence (Yes), n=936 | P value |
|----------------------------------------|--------------------------------|--------------------------------|----------|
| Age, years, median (IQR)               | 62 (54.70)                     | 64 (56.73)                     | <0.0001  |
| Male, n (%)                            | 6574 (68.7)                    | 601 (64.2)                     | 0.004    |
| BMI, kg/m², median (IQR)               | 24.5 (22.6,26.6)               | 24.5 (22.6,26.7)               | 0.85     |
| Previous lipid-lowering agents use, n (%) | 1095 (11.5)                 | 130 (13.9)                     | 0.03     |
| Medical history, n (%)                 |                               |                               |          |
| Hypertension                           | 6003 (62.8)                    | 603 (64.4)                     | 0.32     |
| Diabetes mellitus                      | 2247 (23.5)                    | 264 (28.2)                     | 0.001    |
| Dyslipidemia                           | 791 (8.3)                      | 73 (7.8)                       | 0.62     |
| Cardiocerebrovascular diseases         | 2765 (28.9)                    | 379 (40.5)                     | <0.0001  |
| Index event, n (%)                     | 8882 (92.9)                    | 902 (96.4)                     | <0.0001  |
| TOAST classification, n (%)            | 2336 (24.4)                    | 319 (34.1)                     | <0.0001  |
| IS                                     | 8681 (7.1)                     | 24 (3.6)                       |          |
| Fasting plasm glucose                  |                                |                               |          |
| LAA                                    | 148 (135.164)                  | 51 (138.168)                   | <0.0001  |
| Non-LAA                                | 5.50 (4.88,6.82)               | 5.81 (5.05,7.64)               | <0.0001  |
| Lipid-lowering drugs                   |                                |                               |          |
| LDL-C                                  | 2.30 (1.72,2.97)               | 2.38 (1.78,3.06)               | 0.009    |
| HDL-C                                  | 1.07 (0.90,1.29)               | 1.07 (0.89,1.26)               | 0.18     |
| TG                                     | 1.37 (1.03,1.87)               | 1.34 (1.02,1.88)               | 0.28     |
| hsCRP at baseline, mg/L, median (IQR)  | 1.72 (0.81,4.50)               | 2.37 (0.96,6.21)               | <0.0001  |
| IL-6, ng/L, median (IQR)               | 2.59 (1.56,4.90)               | 3.20 (1.84,6.58)               | <0.0001  |

**Abbreviations:** IQR = interquartile range; LAA = large artery atherosclerosis; non-LAA = non-large artery atherosclerosis; BP = blood pressure; FPG = fasting plasma glucose; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6.

BMI is the weight in kilograms divided by the square of the height in meters. TOAST denotes stroke etiology classification criteria according to the Trial of Org 10172 in Acute Stroke Treatment.

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**Supplemental Fig. 2.** Hazard Ratio for Incidence Rate of Recurrent Ischemic Stroke in Subgroups

*Adjusted for age, sex, BP at admission, FPG at baseline, medical history of diabetes mellitus, cardioscerebrovascular diseases, TOAST classification, medication use of lipid-lowering drugs and antplatelet agents.

**Abbreviations:** LDL-C: low-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein; LAA: large-artery atherosclerosis; HR: hazard ratio; CI: confidence interval.