Soluble CD40L Is a Useful Marker to Predict Future Strokes in Patients With Minor Stroke and Transient Ischemic Attack

Jiejie Li, MD, PhD*; Yilong Wang, MD, PhD*; Jinx Lin, PhD; David Wang, DO; Anxin Wang, MD; Xingquan Zhao, MD, PhD; Liping Liu, MD, PhD; Chunxue Wang, MD, PhD; Yongjun Wang, MD; on behalf of the CHANCE Investigators

Background and Purpose—Elevated soluble CD40 ligand (sCD40L) was shown to be related to cardiovascular events, but the role of sCD40L in predicting recurrent stroke remains unclear.

Methods—Baseline sCD40L levels were measured in 3044 consecutive patients with acute minor stroke and transient ischemic attack, who had previously been enrolled in the Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events (CHANCE) trial. Cox proportional-hazards model was used to assess the association of sCD40L with recurrent stroke.

Results—Patients in the top tertile of sCD40L levels had increased risk of recurrent stroke comparing with those in the bottom tertile, after adjusted for conventional confounding factors (hazard ratio, 1.49; 95% confidence interval, 1.11–2.00; P=0.008). The patients with elevated levels of both sCD40L and high-sensitive C-reactive protein also had increased risk of recurrent stroke (hazard ratio, 1.81; 95% confidence interval, 1.23–2.68; P=0.003).

Conclusions—Elevated sCD40L levels independently predict recurrent stroke in patients with minor stroke and transient ischemic attack.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00979589.
(Stroke. 2015;46:1990-1992. DOI: 10.1161/STROKEAHA.115.008685.)

Key Words: CD40 ligand ◼ ischemic attack, transient ◼ prognosis ◼ stroke

CD40 ligand (CD40L) plays a role in atherosclerotic plaque instability.1 Soluble CD40L (sCD40L) was released from activated platelet and shown to be a predictor of first2 and subsequent cardiovascular events.3 Recently, a genetic study indicated that CD40-1C>T polymorphisms was associated with the risk of reocclusion after successful fibrinolytic therapy in patients with stroke,4 suggesting that sCD40L might be associated with prognosis of stroke. However, the role of sCD40L in predicting recurrent stroke remained unclear.

The Clopidogrel in High-Risk Patients With Acute Nondisabling Cerebrovascular Events (CHANCE) trial compared efficacy of clopidogrel plus aspirin or aspirin alone.5 Serum samples were collected 24±12 hours after randomization. The primary outcome was stroke within 90-day follow-up period. The CHANCE protocol was approved by the ethics committee at each study center.

Measurements of Biomarkers
sCD40L levels were determined via an ELISA kit (catalogue number BMS239; eBioscience, Vienna, Austria). High-sensitive C-reactive

Received January 12, 2015; final revision April 13, 2015; accepted April 21, 2015.
From the Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China (J.L., Yilong Wang, J.L., A.W., X.Z., L.L., C.W., Yongjun Wang); China National Clinical Research Center for Neurological Diseases, Beijing. China (Yongjun Wang); Center of Stroke, Beijing Institute for Brain Disorders, Beijing, China (Yongjun Wang); Beijing Key Laboratory of Translational Medicine for Cerebrovascular Disease, Beijing, China (Yongjun Wang); and Illinois Neurological, Institute Stroke Network, Sisters of the Third Order of St. Francis Healthcare System, University of Illinois College of Medicine, Peoria (D.W.).
*Dr Li and Wang contributed equally.
The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.115.008685/-/DC1.

Reprint requests to Yongjun Wang, MD, No. 6 Tiantanxili, Beijing 100050, China. E-mail yongjunwang1962@gmail.com
© 2015 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.115.008685

1990
protein (hsCRP) was measured in the clinical laboratory in Tiantan hospital. All measurements were performed by laboratory personnel blinded to the study status.

Statistical Analysis
Proportions were used for categorical variables. Medians with interquartile ranges were used for continuous variables because of skewed distribution. Categorical and continuous variables were compared with the \( \chi^2 \) statistics and Kruskal–Wallis test, respectively. The relationship of sCD40L and outcome was investigated with Cox proportional-hazards model. A 2-sided \( P \) value of <0.05 was considered to be statistically significant.

Results
Patient Characteristics
There were 73 (64%) prespecified centers voluntarily participated in the biomarker substudy, enrolling 3044 consecutive patients.

Characteristics of patients are shown in Table I in the online-only Data Supplement. Patients in the top tertile of sCD40L levels were younger, likely had history of hypercholesterolemia, and higher baseline levels of low-density lipoprotein-cholesterol, triglycerides, hsCRP, and leukocyte count.

sCD40L and Recurrence of Stroke
Patients in the top tertile of sCD40L levels had increased risk of recurrent stroke (Figure I in the online-only Data Supplement). The increased risk remained after adjusted for other confounding factors (Table). The hazard ratios increased with rising tertiles (Table). Similar results were found when sCD40L was assessed as a continuous variable (Table). Besides sCD40L, the factors of age, history of hypertension and diabetes mellitus, baseline National Institutes of Health Stroke Scale, baseline low-density lipoprotein-cholesterol levels, and dual antiplatelet therapy also predicted subsequent strokes (Figure II in the online-only Data Supplement).

sCD40L was interacted with hsCRP in its effect on recurrent stroke (\( P=0.01 \)). Patients were subsequently divided into 6 groups according to tertiles of sCD40L levels and concentrations of hsCRP either >3 or \( \leq 3 \) mg/L. The patients with elevated levels of both sCD40L and hsCRP also had increased risk of recurrent stroke (hazard ratio, 1.81; 95% confidence interval, 1.23–2.68; \( P=0.003 \); Figure III in the online-only Data Supplement).

Discussion
Although the role of sCD40L in predicting cardiovascular events has been well elucidated before, the association between sCD40L and stroke was still unclear.6,7 One potential reason for this apparent discrepancy may relate to the use of combined vascular events as the end point, which would make the individual association of sCD40L with stroke not as apparent. On the contrary, most of these researches have focused on the first stroke.

sCD40L played a role in atherosclerotic plaque instability1 and platelet activation.8 Our results showed sCD40L predicted recurrent stroke, which indicated that sCD40L may represent another approach other than conventional risk factors to stratify the risk of recurrent stroke. Furthermore, it has been noted well that ischemic events still occurred in some patients, despite sustained standard secondary preventive therapy, indicating other therapeutic strategies should be implemented. Our findings might suggest sCD40L as a target for studies targeting the inflammatory cascade for stroke prevention. hsCRP was another inflammatory marker associated with prognosis of stroke; however, power was limited to find an independent effect of the combination of these 2 biomarkers because of overlapped confidence intervals in the current study.

Our study had some limitations. First, only baseline levels of sCD40L were obtained and the fluctuation of sCD40L could not be completely eliminated. Second, we did not adjust statin dose because of lack of the data, which was shown to be associated with stroke risk.10 Third, the data on stroke subtypes were absent. These should be considered in the further study.

In conclusion, this substudy of CHANCE trial suggested that baseline sCD40L could serve as a prognostic factor of future recurrent stroke in patients with acute minor ischemic

Table. Risk of Recurrent Stroke According to Levels of sCD40L

<table>
<thead>
<tr>
<th>Tertile</th>
<th>sCD40L levels†</th>
<th>No. of Events (%)</th>
<th>HR (95% CI)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1</td>
<td>78 (7.7%)</td>
<td>1 (reference)</td>
<td>0.020</td>
<td>0.017</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>107 (10.5%)</td>
<td>1.38 (1.03–1.85)</td>
<td>0.029</td>
<td>1.43 (1.07–1.92)</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>114 (11.2%)</td>
<td>1.49 (1.11–1.98)</td>
<td>0.007</td>
<td>1.49 (1.11–2.00)</td>
</tr>
<tr>
<td>Continuous model‡</td>
<td>1.04 (1.01–1.07)</td>
<td>0.007</td>
<td>1.03 (1.01–1.06)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; HR, hazard ratio; and sCD40L, soluble CD40 ligand.

*Model 1, unadjusted; model 2, adjusted for age, sex, body mass index, current or previous smoking, medical history of hypertension, diabetes mellitus, hypercholesterolemia, ischemic stroke, transient ischemic attack, myocardial infarction, angina, known atrial fibrillation or flutter and valvular heart disease, randomized treatment of aspirin monotherapy or dual antiplatelet therapy, National Institutes of Health Stroke Scale score at 1 day after randomization, qualifying event and baseline levels of serum lipids (low-density lipoprotein-cholesterol, high-density lipoprotein, and triglycerides); model 3, adjusted for all factors in model 2, baseline high-sensitive C-reactive protein levels and baseline leukocyte count; model 4, adjusted for all factors in model 2 and the use of lipid-lowering agents, hypoglycemic agents or antihypertension agents during 90-day follow-up period.

†sCD40L level was divided by tertiles: tertile 1, sCD40L <6.21 ng/mL; tertile 2, sCD40L 6.21 to 9.32 ng/mL; and tertile 3, sCD40L \( \geq 9.32 \) ng/mL.

‡Hazard ratios correspond to per unit increment of sCD40L value (ng/mL).
stroke and transient ischemic attack. Analysis of this biomarker may represent another approach to stratify the risk of recurrent stroke.

Sources of Funding
This study was, in part, supported by a grant (No. 81200844 to Dr Li) from The National Natural Science Foundation of China, a grant (No.2014ZZ-10 to Dr Li) from Beijing Postdoctoral Working Funding, grants (Nos 2008ZX09312-008, 2012ZX09303, and 200902004 to Dr Yongjun Wang; No. 2011BAI08B02 to Dr Yilong Wang) from the Ministry of Science and Technology of the People’s Republic of China, a grant (No. D131100005313003 to Dr Yongjun Wang) from Beijing Biobank of Cerebral Vascular Disease, a grant (No. 81471211 to Dr Yongjun Wang) from the National Natural Science Foundation of China.

Disclosures
None.

References
Soluble CD40L Is a Useful Marker to Predict Future Strokes in Patients With Minor Stroke and Transient Ischemic Attack

Jiejie Li, Yilong Wang, Jinxin Lin, David Wang, Anxin Wang, Xingquan Zhao, Liping Liu, Chunxue Wang and Yongjun Wang

on behalf of the CHANCE Investigators

*Stroke*. 2015;46:1990-1992; originally published online May 26, 2015; doi: 10.1161/STROKEAHA.115.008685

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://stroke.ahajournals.org/content/46/7/1990

Data Supplement (unedited) at:

http://stroke.ahajournals.org/content/suppl/2015/05/26/STROKEAHA.115.008685.DC1

**Permissions**: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints**: Information about reprints can be found online at:

http://www.lww.com/reprints

**Subscriptions**: Information about subscribing to *Stroke* is online at:

http://stroke.ahajournals.org/subscriptions/
Table I. Baseline Characteristics and Medication within 90-day Follow-up Period According to Tertiles of sCD40L

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>sCD40L Level, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 1 (&lt;6.21)</td>
</tr>
<tr>
<td></td>
<td>n=1014</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>63 (56 to 72)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>24 (23 to 26)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>321 (31.7)</td>
</tr>
<tr>
<td>Medical history, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>184(18.1)</td>
</tr>
<tr>
<td>TIA</td>
<td>32(3.2)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>22(2.2)</td>
</tr>
<tr>
<td>Angina</td>
<td>30(3.0)</td>
</tr>
<tr>
<td>Known atrial fibrillation or flutter</td>
<td>16(1.6)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>87(8.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>680(67.1)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>211(20.8)</td>
</tr>
<tr>
<td>Current or previous smoking, No. (%)</td>
<td>450(44.4)</td>
</tr>
<tr>
<td>NIHSS, median (IQR) *</td>
<td>2 (0 to 2)</td>
</tr>
<tr>
<td>Baseline biomarkers, median (IQR)</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>3.0 (2.4 to 3.7)</td>
</tr>
<tr>
<td>HDL</td>
<td>1.2 (1.0 to 1.5)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.4 (1.0 to 1.9)</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.6 (0.8 to 3.7)</td>
</tr>
<tr>
<td></td>
<td>6.4(5.4 to 7.7)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Medication within 3 months, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Anti-hypertensive agent</td>
<td>357(35.3%)</td>
</tr>
<tr>
<td>Hypoglycemic agents</td>
<td>129(12.8%)</td>
</tr>
<tr>
<td>Lipid-lowering agents</td>
<td>431(42.7%)</td>
</tr>
</tbody>
</table>

sCD40L indicates soluble CD40 ligand; IQR, interquartile range; BMI, body-mass index; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; LDL-C, LDL cholesterol; HDL, HDL cholesterol; and hsCRP, high sensitivity C reactive protein.
Supplemental Figures

Figure I. Kaplan–Meier curves showing the probability of survival free of stroke during 90-day follow-up according to tertiles of sCD40L levels. sCD40L level was divided by tertiles: tertile 1, sCD40L <6.21 ng/ml; tertile 2, sCD40L 6.21 to 9.32 ng/ml; tertile 3, sCD40L ≥9.32 ng/ml. The inset shows the same data on an enlarged segment of the y axis.
Figure II. Factors associated with recurrent stroke during 90-day follow-up. sCD40L tertiles, age, sex, body-mass index (BMI), current or previous smoking, medical history of hypertension, diabetes mellitus, hypercholesterolemia, ischemic stroke, TIA, myocardial infarction, angina, known atrial fibrillation or flutter and valvular heart disease, randomized treatment of aspirin monotherapy or dual antiplatelet therapy, NIHSS score at 1 day after randomization, qualifying event, baseline levels of serum lipids (LDL-c, HDL and triglycerides), baseline hsCRP levels and baseline leukocyte count were included in the Cox proportional-hazards model with time-to-event for stroke as the dependent variable.
Figure III. Combination of sCD40L with hsCRP predicted outcomes. Patients were divided into 6 groups according to tertiles of sCD40L levels and concentrations of hsCRP either > 3 or ≤ 3 mg/L, the cut point recommended by the Centers for Disease Control and the American Heart Association for high risk category to develop cardiovascular events (low risk, <1.0 mg/L; average risk, 1 to 3 mg/L; and high risk, >3 mg/L)\(^1\) and associated with increased risk of recurrent stroke\(^2\): group 1: sCD40L < 6.21 ng/mL and hsCPR ≤ 3 mg/L; group 2: sCD40L 6.21 to 9.32 ng/mL and hsCPR ≤ 3 mg/L; group 3: sCD40L ≥ 9.32 ng/mL and hsCPR ≤ 3 mg/L; group 4: sCD40L < 6.21 ng/mL and hsCPR > 3 mg/L; group 5: sCD40L 6.21 to 9.32 ng/mL and hsCPR > 3 mg/L; group 6: sCD40L ≥ 9.32 ng/mL and hsCPR > 3 mg/L. Group 1 was assigned as a reference category with a relative risk of 1. The Cox proportional-hazards model was adjusted for age, sex, BMI, current or previous smoking, medical history of hypertension, diabetes mellitus, hypercholesterolemia, ischemic stroke, TIA, myocardial infarction, angina, known atrial fibrillation or flutter and valvular heart disease, randomized treatment of aspirin monotherapy or dual antiplatelet therapy, NIHSS score at 1 day after randomization, qualifying event and baseline levels of serum lipids (LDL-c, HDL and triglycerides). * p<0.05 vs patients in group 1.

Reference


The CHANCE Investigators
Yongjun Wang, MD, PhD (BeijingTiantan Hospital, Principal Investigator); S.Claiborne Johnston, MD, PhD (Departments of Neurology and Epidemiology, University of California, San Francisco, USA, Co - Principal Investigator); Yilong Wang, MD, PhD (BeijingTiantan Hospital, Executive Committee); Xingquan Zhao, MD, PhD (BeijingTiantan Hospital, Site Investigator); Zhimin Wang, MD, PhD (Taizhou First People's Hospital, Site Investigator); Haiqin Xia, MD, PhD (Taiyuan Iron And Steel [Group] Co., Ltd., General Hospital, Site Investigator); (Dagang Oilfield Gengeal Hospital, Site Investigator); Guiru Zhang, MD, PhD (Penglai People's Hospital, Site Investigator); Xudong Ren, MD, PhD (The Third People's Hospital Of Datong, Site Investigator); Chunling Ji, MD, PhD (The Fourth Central Hospital Of Tianjin, Site Investigator); Guohua Zhang, MD, PhD (The Second Hospital Of Hebei Medical University, Site Investigator); Jianhua Li, MD, PhD (The First Hospital Of Fangshan District, Beijing, Site Investigator); Bohua Lu, MD, PhD (Beijing Puren Hospital, Site Investigator); Liping Wang, MD, PhD (Tianjin Ninghe District Hospital, Site Investigator); Shutao Feng, MD, PhD (The People's Hospital Of Zhengzhou, Site Investigator); Dali Wang, MD, PhD (Affiliated Hospital Of North China Coal Medical College, Site Investigator); Weiguo Tang, MD, PhD (Zhejiang Zhoushan Hospital, Site Investigator); Juntao Li, MD, PhD (Han Dan Central Hospital, Site Investigator); Hongtian Zhang, MD, PhD (Zhecheng People's Hospital, Site Investigator); Guangliai Li, MD, PhD (Shanxi Medical University Second Hospital, Site Investigator); Baojun Wang, MD, PhD (Baotou Central Hospital, Site Investigator); Yuhua Chen, MD, PhD (The General Hospital Of Changjiang River Shipping, Site Investigator); Ying Lian, MD, PhD (Dalian Economic And Technological Development Zone Hospital, Site Investigator); Junfang Teng, MD, PhD (First Neurology Department, Affiliated Hospital Of North China Coal Medical College, Site Investigator); Li Sun, MD, PhD (Qingdao Central Hospital, Site Investigator); Dong Wang, MD, PhD (Baogang Hospital, Site Investigator); Liying Hou, MD, PhD (ChangZhi City People's Hospital Of Shanxi Province, Site Investigator); Dongcai Yuan, MD, PhD (HaLixun International Peace Hospital, Site Investigator); Yongliang Cao, MD, PhD (People's Hospital Of Linzi District, Zibo, Site Investigator); Hui Li, MD, PhD (Yantai City Yantai Mountain Hospital, Site Investigator); Xiuge Tan, MD, PhD (Beijing Pinggu District Hospital, Site Investigator); Huicong Wang, MD, PhD (Taiyuan Central Hospital, Site Investigator); Haisong Du, MD, PhD (Chengde Central Hospital, Site Investigator); Mingyi Liu, MD, PhD (Shijiazhuang Central Hospital, Site Investigator); Suping Wang, MD, PhD (First Neurology Department, Dalian Municipal Central Hospital, Site Investigator); Qiuwu Liu, MD, PhD (Xian 141 Hospital, Site Investigator); Zhong Zhang, MD, PhD
Haidian Hospital, Site Investigator); Quping Ouyang, MD, PhD (Beijing Shunyi District Hospital, Site Investigator); Jingbo Zhang, MD, PhD (Dalian Third Municipal Hospital, Site Investigator); Anding Xu, MD, PhD (The First Affiliated Hospital Of Jinan University, Site Investigator); Xiaokun Qi, MD, PhD (Navy General Hospital Of P.L.A, Site Investigator); Lei Wang, MD, PhD (Beijing Second Artillery General Hospital, Site Investigator); Fuming Shi, MD, PhD (Beijing Daxing District Hospital, Site Investigator); Fuqiang Guo, MD, PhD (Sichuan Province People's Hospital, Site Investigator); Jianfeng Wang, MD, PhD (Dalian Municipal Central Hospital, Site Investigator); Fengli Zhao, MD, PhD (The Second Hospital In Baoding, Site Investigator); Ronghua Dou, MD, PhD (The Hospital Combine Traditional Chinese And Western Medicine In Cangzhou, Site Investigator); Dongning Wei, MD, PhD (The 309th Hospital Of P.L.A, Site Investigator); Qingwei Meng, MD, PhD (Liangxiang Hospital Of Fangshan District, Beijing, Site Investigator); Yilu Xia, MD, PhD (Huaxin Hospital First Hospital Of Tsinghua University, Site Investigator); Shimin Wang, MD, PhD (Tianjin Huanhu Hospital, Site Investigator); Zhihui Liu, MD, PhD (Beijing University Of Chinese Medicine East Hospital, Site Investigator); Wei Chen, MD, PhD (Zhejiang University Of Chinese Medicine Affiliated First Hospital, Site Investigator); Xiaodong Yuan, MD, PhD (Affiliated Hospital Of Kailuan Company Ltd, Site Investigator); Zhihui Liu, MD, PhD (Affiliated Hospital Of Weifang Medical University, Site Investigator); Guozhong Li, MD, PhD (The First Hospital Of Harbin Medical University, Site Investigator); Xiaohong Li, MD, PhD (Dalian Friendship Hospital, Site Investigator); Tingchen Tian, MD, PhD (Tianjin Dagang Hospital, Site Investigator).