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ORIGINAL RESEARCH

A Score of Low-Grade Inflammation for Predicting Stroke Recurrence in Patients with Ischemic Stroke

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Background and Purpose: The impact of low-grade inflammation (LGI) on stroke recurrence has not been well studied yet. We aimed to evaluate the association between LGI and stroke recurrence in patients with ischemic stroke.

Methods: Patients with first-ever ischemic stroke diagnosed within 72 hours of symptoms onset were consecutively recruited from the Nanjing Stroke Registry Program. C-reactive protein (CRP) level, white blood cell (WBC) count, platelet (PLT) count, and neutrophil-to-lymphocyte ratio (NLR) were conceived as low-grade inflammation biomarkers and combined into a standardized LGI score. The association of LGI score with the risk of stroke recurrence was analyzed with multivariate Cox regression models. Analyses accounting for the competing risk of stroke recurrence and death were also performed.

Results: Of the 1214 patients studied (median age, 61 years; male, 71.6%), 177 (14.6%) patients experienced a recurrent stroke with a median follow-up of 23.0 (interquartile ranges, 14.5–34.0) months. Patients with stroke recurrence had a higher LGI score (median, 3 versus −2; $P < 0.001$) than those without recurrence. The univariate analysis indicated that patients with LGI scores in the fourth quartile were more likely to have a stroke recurrence (hazard ratios [HR], 4.312; 95% confidence interval [CI], 2.675–6.952; $P < 0.001$). After adjusting for potential confounders, the association remained significant in the multivariate Cox regression model (HR, 4.080; 95% CI, 2.420–6.879; $P < 0.001$). Competing risks model and sensitivity analysis further confirmed this conclusion.

Conclusion: This study showed that an elevated LGI score was associated with a higher risk of stroke recurrence, independent of other vascular risk factors.

Keywords: low-grade inflammation, stroke recurrence, ischemic stroke, predictor

Introduction

In most countries, the incidence of ischemic stroke is increasing with the age of the general population.[1,2] A recurrent stroke occurs more frequently during the first years after stroke, with estimated frequency ranging between 7.1% and 26.0%, depending on the population studied and the length of follow-up.[3–5] Furthermore, stroke recurrence is more fatal and disabling than the first-ever stroke.[6] Thus, early identification and management of the high risk of recurrent stroke patients are of great significance.

Low-grade inflammation (LGI) has been reported to be associated with several chronic conditions including cardiovascular disease, cancer, and neurodegenerative disease.[7–9] Due to the sensitivity to lifestyle modifications, LGI also emerged as an important target for disease prevention.[10] Several biomarkers, both circulating (ie,

4605

C-reactive protein [CRP]) and cellular (ie, platelet [PLT] and white blood cell [WBC] count) have been recognized as indicators of LGI.[11,12] The CAPRIE study (Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events) demonstrated that increased leukocyte count was related to recurrent ischemic stroke.[13] However, several large-scale epidemiological studies on CRP level and recurrent vascular events yielded inconsistent results, some showing significant positive associations between CRP and stroke recurrence,[14] whereas others indicated no association.[15] Recently, the neutrophil-to-lymphocyte ratio (NLR) was also shown to be a reliable marker of cellular inflammation.[16] Considering that these LGI markers do not occur separately in a patient, a comprehensive approach that takes into account the possible synergistic effects might better illustrate the LGI status. Meanwhile, the association of LGI status with recurrent stroke has not been fully investigated.

Thus, the study aimed to use a predefined composite LGI score based on the 4 abovementioned individual LGI markers and to investigate its relationship with stroke recurrence in a cohort of patients who initially presented with first-ever ischemic stroke.

Materials and Methods
Study Population
Patients with first-ever ischemic stroke were consecutively enrolled in the Nanjing Stroke Registry Program (NSRP) from January 2012 to December 2016. It is a prospective, ongoing, hospital-based stroke registry inaugurated in July 2002. The detailed protocol for this registry has been published previously.[5,17] Patients were enrolled if they met the following criteria: (a) age ≥18 years; (b) symptoms from onset to admission <72 hours. The excluded criteria were as follows: (a) patients experienced infection within recent 30 days; (b) suffered from previous neurological diseases such as cerebral hemorrhage, severe head trauma, and central nervous system infection; (c) had severe hepatic or renal disease, hematological disease, malignant tumor, autoimmune disease, and immunosuppressive therapy. Investigation on the NSRP was approved by the Ethics Review Board of Jinling Hospital (approval number 2010NLY-018). All participants or their relatives signed written consent forms. This study was conducted in accordance with the Declaration of Helsinki.

Data Collection
Demographic characteristics, clinical variables, vascular risk factors, laboratory results, and stroke etiology were all collected and evaluated. Baseline stroke severity was assessed by the National Institute of Health Stroke Scale (NIHSS) score.[18] Stroke etiology was classified according to the Trial of ORG 10172 in acute stroke treatment classification.[19] Subjects were defined as smokers if they smoked >400 cigarettes in their lifetime.[20] They were further classified as former smokers if they had quit smoking for at least 30 days before the index stroke, quitters who had quit for at least 1 month and kept cessation at the first follow-up, and the persistent who continued smoking at the first follow-up.[21] The educational levels were categorized into four groups according to the educational years: 0–6 years (primary school), 6–9 years (middle school), 9–12 years (high school), and >12 (university or higher education).

Measurement of LGI
Blood samples were obtained from each subject within 24 h after admission. The CRP level, WBC count, PLT count, and NLR were measured and combined into the LGI score. For calculating the LGI score, 10-tiles of each biomarker level (CRP, WBC, PLT, NLR) were generated. Being in the highest deciles (>6) of all individual LGI markers gave a score that increased from 1 to 4, while being in the lowest deciles (<5) was negatively scored from −4 to −1. The middle deciles (5 and 6) were assigned a score of 0. The LGI score was the sum of the 4 markers and ranged between −16 and 16 (Figure 1).[11]

Endpoint and Follow-Up Assessments
The primary endpoint was the fatal or nonfatal stroke recurrence after 3 months of the index stroke, which is defined as a new neurological deficit or a sudden deterioration of a previous deficit lasting for >24 h, in the absence of apparent causes other than that of vascular origin.[22] The follow-up was initiated at the 3, 6, 12 months after the index stroke and annually thereafter and extended to the date of the first recurrent stroke or censoring via telephone interviews or clinical visits.[5] Patients were censored at the date of the emigration from this study, the date of death from other causes, or the end of the assigned follow-up period. The death was verified by examining the death certificates and medical records.
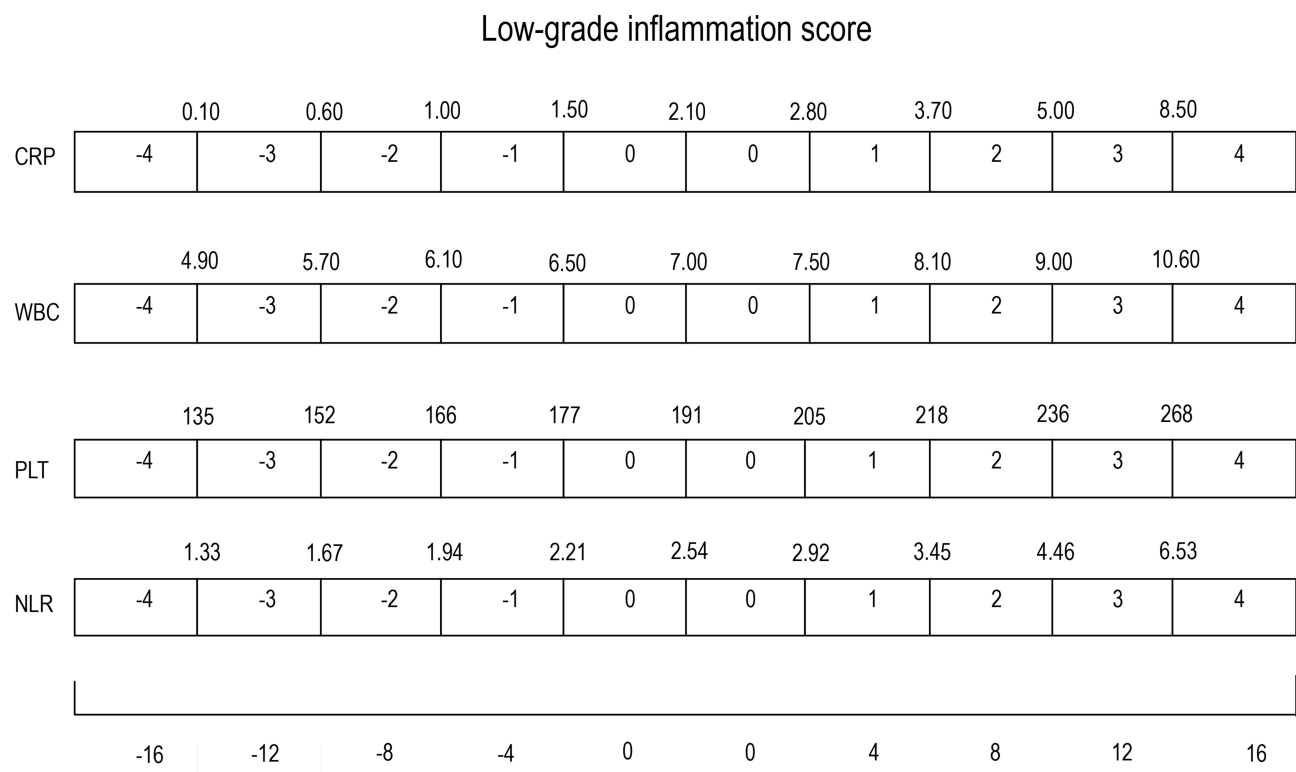
Low-grade inflammation score

Figure 1 The calculation of low-grade inflammation score.
Abbreviations: CRP, C-reactive protein; WBC, white blood cell; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 24.0 (SPSS Inc., Chicago, IL), and R statistical software, version 4.0.0. (R Foundation, Vienna, Austria). Categorical variables were expressed as numbers (percentages) and analyzed with Chi-square or Fisher's exact test. Continuous variables were presented as mean (standard deviations) or median (interquartile ranges, IQR) and analyzed by t-test, Mann–Whitney U-test, one-way analysis of variance, and Kruskal–Wallis test as appropriate.

The collinearity between candidate variables was examined using variance inflation factors before developing the Cox proportional hazards models, which were performed to estimate hazard ratios (HR) with a 95% confidence interval (CI) concerning stroke recurrence. Model 1 adjusted for age and sex, and model 2 adjusted for age, sex, and factors with a P-value <0.1 in the univariate analysis, of which the predictive capacity was measured by concordance index (c-index). The receiver operating characteristics (ROC) curve was performed to examine the discrimination of the LGI score and the individual biomarkers of the score. The cumulative incidence curves of stroke recurrence were also plotted according to the LGI score using Kaplan–Meier survival analysis. Moreover, the higher LGI score is a significant risk factor for death and the Fine and Gray's proportional subdistribution hazards regression models were used to examine the possible influence of death on the association between LGI score and risk of recurrent stroke.[23]

Meanwhile, the LGI score might differ according to sex, age, cardiovascular risk factors, blood pressure at admission, and baseline stroke severity, which are also important factors of stroke recurrence. Thus, sensitivity analysis was performed by stratifying participants into different subgroups, and tests of interaction were also performed in terms of the factors aforementioned.

Moreover, the associations of LGI scores with different stroke subtypes were further analyzed using Fisher's exact test with the Bonferroni methods. A two-tailed P-value <0.05 was considered statistically significant.

Results

Of the 1214 participants included in this study, the median age was 61 years and 869 (71.6%) were male. The median levels of CRP, WBC count, PLT count, and NLR were

2.1 mg/L, $7.0×10^9$/L, $191×10^9$/L, and 2.5, respectively. During an average follow-up of 23.0 (IQR, 14.5–34.0) months, 177 (14.6%) patients experienced a recurrent stroke, of which 159 patients with ischemic stroke and 18 with hemorrhagic stroke.

Table 1 shows the clinical characteristics of the study sample stratified by the quartile of LGI score. Compared with patients in the first quartile of LGI scores, those with increasing LGI scores had a higher ratio of large-artery atherosclerosis ($P=0.003$), a lower education level ($P<0.001$), and a higher baseline NIHSS score ($P<0.001$).

Table 2 illustrates the demographic characteristics, clinical data, and laboratory data according to the presence or absence of stroke recurrence. Patients with recurrence were older (median age, 66 versus 60 years, $P<0.001$), had a higher prevalence of large artery atherosclerosis

Table I Baseline Data According to the Quartile of Low-Grade Inflammation Score

Variables	All Patients n = 1214	Q1 (≤-5) n = 335	Q2 (−4 to −1) n =278	Q3 (0 to 4) n = 331	Q4 (>4) n =270	P for Trend
Age, years, median [IQR]	61 (52, 68)	62 (54, 68)	61 (52, 67)	61 (51, 69)	59 (49, 67)	0.052
Male, n (%)	869 (71.6)	234 (69.9)	205 (73.7)	233 (70.4)	197 (73.0)	0.656
BMI, kg/m^2, median [IQR]	24.5 (22.5, 26.6)	24.4 (22.5, 26.3)	24.7 (22.7, 26.8)	24.5 (22.9, 27.0)	24.7 (22.9, 26.3)	0.366
Baseline NIHSS score, median [IQR]	4.0 (2.0, 8.0)	3.0 (1.0, 6.0)	3.0 (1.0, 6.0)	4.0 (2.0, 8.0)	5.0 (2.0, 10.0)	<0.001
Stroke subtypes, n (%)						0.003
LAA	469 (38.6)	117 (34.9)	99 (35.6)	134 (40.5)	119 (44.1)	
CE	113 (9.3)	30 (9.0)	23 (8.3)	30 (9.1)	30 (11.1)	
SAA	248 (20.4)	87 (26.0)	70 (25.2)	59 (17.8)	32 (11.9)	
Others	384 (31.6)	101 (30.1)	86 (30.9)	108 (32.6)	89 (33.0)	
Education years, n (%)						<0.001
0–6	314 (25.9)	98 (29.3)	74 (26.6)	93 (28.1)	49 (18.1)	
6–9	619 (51.0)	152 (45.4)	127 (45.7)	172 (52.0)	168 (62.2)	
9–12	143 (11.8)	43 (12.8)	42 (15.1)	43 (13.0)	15 (5.6)	
>12	138 (11.4)	42 (12.5)	35 (12.6)	23 (6.9)	38 (14.1)	
Vascular risk factors, n (%)						
Hypertension	848 (69.9)	244 (72.8)	186 (66.9)	234 (70.7)	184 (68.1)	0.385
Diabetes mellitus	355 (29.2)	99 (29.6)	78 (28.1)	98 (29.6)	80 (29.6)	0.970
Hyperlipidemia	123 (10.1)	30 (9.0)	31 (11.2)	37 (11.2)	25 (9.3)	0.694
Coronary heart disease	86 (7.1)	29 (8.7)	17 (6.1)	25 (7.6)	15 (5.6)	0.436
Smoking status, n (%)						0.316
Nonsmokers	520 (42.8)	157 (46.9)	112 (40.3)	135 (40.8)	116 (43.0)	
Former smokers	115 (9.5)	36 (10.7)	21 (7.6)	37 (11.2)	21 (7.8)	
Quitters	325 (26.8)	84 (25.1)	81 (29.1)	83 (25.1)	77 (28.5)	
Persistent smokers	254 (20.9)	58 (17.3)	64 (23.0)	76 (23.0)	56 (20.7)	
Baseline SBP, mmHg, median [IQR]	140 (130, 152)	137 (128, 150)	142 (130, 153)	140 (130, 154)	140 (130, 152)	0.002
Baseline DBP, mmHg, median [IQR]	80 (75, 90)	80 (74, 85)	80 (75, 90)	80 (76, 90)	80 (75, 90)	0.002
Blood glucose, mmol/L, median [IQR]	5.8 (4.9, 7.5)	5.4 (4.6, 6.7)	5.6 (4.8, 7.2)	6.1 (5.0, 8.0)	6.3 (5.3, 7.9)	<0.001
CRP, mg/L, median [IQR]	2.1 (0.7, 4.2)	0.8 (0.1, 2.1)	1.5 (0.3, 2.9)	2.7 (1.2, 4.4)	4.9 (2.8, 8.9)	<0.001
WBC count, 10^9/L, median [IQR]	7.0 (5.9, 8.5)	5.6 (4.7, 6.3)	6.6 (5.9, 7.5)	7.6 (6.6, 8.7)	9.5 (8.1, 11.2)	<0.001
PLT count, 10^9/L, median [IQR]	191 (159, 227)	163 (139, 188)	191 (165, 219)	196 (165, 232)	228 (196, 266)	<0.001
NLR, median [IQR]	2.5 (1.8, 3.9)	1.8 (1.4, 2.4)	2.2 (1.7, 2.8)	2.9 (2.1, 4.0)	5.0 (3.2, 8.2)	<0.001
Stroke recurrence, n (%)	177(14.6)	22 (6.6)	26 (9.4)	57 (17.2)	72 (26.7)	<0.001

Abbreviations: IQR, interquartile ranges; BMI, body mass index; NIHSS, National Institute of Health Stroke Scale; LAA, large-artery atherosclerosis; CE, cardioembolism; SAA, small-vessel occlusion; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; WBC, white blood cell; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio.

Table 2 Comparison of Baseline Data According to Patients with and without Recurrence

Variables	Recurrence n =177	Without Recurrence n = 1037	P	Unadjusted HR (95% CI)	P
Age, years, median [IQR]	66 (57, 73)	60 (51, 67)	<0.001	1.035 (1.021–1.049)	<0.001
Male, n (%)	132 (74.6)	737 (71.1)	0.339	1.153 (0.822–1.618)	0.408
BMI, kg/m2, median [IQR]	24.5 (23.1, 26.7)	24.6 (22.5, 26.6)	0.499	1.006 (0.960–1.055)	0.793
Baseline NIHSS score, median [IQR]	4.0 (2.0, 8.0)	4.0 (2.0, 7.0)	0.179	1.017 (0.993–1.041)	0.164
Stroke subtypes, n (%)			0.087		0.056
LAA	78 (44.1)	391 (37.7)		Reference	
CE	18 (10.2)	95 (9.2)		1.027 (0.615–1.715)	0.918
SAA	24 (13.6)	224 (21.6)		0.537 (0.340–0.848)	0.008
Others	57 (32.2)	327 (31.5)		0.898 (0.638–1.264)	0.538
Education years, n (%)			0.086		0.026
0–6	57 (32.2)	257 (24.8)		Reference	
6–9	85 (48.0)	534 (51.5)		0.691 (0.494–0.967)	0.031
9–12	22 (12.4)	121 (11.7)		0.693 (0.423–1.136)	0.146
>12	13 (7.3)	125 (12.1)		0.434 (0.237–0.794)	0.007
Vascular risk factors, n (%)					
Hypertension	132 (74.6)	716 (69.0)	0.138	1.281 (0.913–1.797)	0.152
Diabetes mellitus	60 (33.9)	295 (28.4)	0.141	1.254 (0.918–1.712)	0.154
Hyperlipidemia	16 (9.0)	107 (10.3)	0.602	0.848 (0.507–1.417)	0.528
Coronary heart disease	11 (6.2)	75 (7.2)	0.626	0.933 (0.507–1.718)	0.823
Smoking status, n (%)			<0.001		<0.001
Nonsmokers	56 (31.6)	464 (44.7)		Reference	
Former smokers	24 (13.6)	91 (8.8)		2.086 (1.293–3.366)	0.003
Quitters	30 (16.9)	295 (28.4)		0.851 (0.546–1.326)	0.476
Persistent smokers	67 (37.9)	187 (18.0)		2.481 (1.740–3.539)	<0.001
Baseline SBP, n (%)					
≥140 mmHg	105 (59.3)	566 (54.6)	0.241	1.237 (0.916–1.670)	0.164
Baseline DBP, n (%)					
≥90mmHg	50 (28.2)	289 (27.9)	0.917	0.984 (0.709–1.364)	0.921
Glucose, mmol/L, median [IQR]	6.3 (5.1, 8.0)	5.8 (4.9, 7.5)	0.050	1.024 (0.980–1.071)	0.287
CRP, mg/L, median [IQR]	4.2 (1.5, 7.9)	2.0 (0.7, 3.9)	<0.001	1.019 (1.008–1.029)	<0.001
WBC count, 10^9/L, median [IQR]	7.7 (6.7, 9.4)	6.9 (5.8, 8.3)	<0.001	1.162 (1.109–1.216)	<0.001
PLT count, 10^9/L, median [IQR]	199 (160, 239)	190 (158, 226)	0.076	1.003 (1.000–1.005)	0.024
NLR, median [IQR]	3.2 (2.1, 6.2)	2.4 (1.8, 3.6)	<0.001	1.092 (1.070–1.114)	<0.001
LGI score, median [IQR]	3 (−1, 8)	−2 (−5, 3)	<0.001	1.103 (1.077–1.130)	<0.001

Abbreviations: HR, hazard ratio; CI, confidence interval; IQR, interquartile ranges; BMI, body mass index; NIHSS, National Institute of Health Stroke Scale; LAA, large-artery atherosclerosis; CE, cardioembolism; SAA, small-vessel occlusion; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; WBC, white blood cell; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; LGI, low-grade inflammation.

numerically (44.1% versus 37.7%, P=0.087), and were more likely to be persistent smokers (37.9% versus 18.0%, P<0.001). The levels of CRP level (median, 4.2 versus 2.0 mg/L, P<0.001), WBC count (median, 7.7 versus 6.9×10^9/L, P<0.001), and NLR (median, 3.2 versus 2.4, P<0.001) were higher in those with recurrence. There was only a numerical difference in the level of PLT count (median, 199 versus 190×10^9/L, P=0.076).

In univariate analysis, the older age (HR, 1.035; 95% CI, 1.021–1.049; *P*<0.001), stroke types (small-vessel occlusion versus large-artery atherosclerosis; HR, 0.537; 95% CI, 0.340–0.848; *P*=0.008), shorter education years (>12 versus 0–6; HR, 0.434; 95% CI, 0.237–0.794; *P*=0.007), smoking status (persistent smokers versus nonsmokers; HR, 2.481; 95% CI, 1.740–3.539; *P*<0.001), high level of CRP (HR, 1.019; 95% CI, 1.008–1.029; *P*<0.001), WBC (HR, 1.162; 95% CI, 1.109–1.216; *P*<0.001), PLT (HR, 1.003; 95% CI, 1.000–1.005; *P*=0.024), and NLR (HR, 1.092; 95% CI, 1.070–1.114; *P*<0.001) were associated with increased risk of recurrent stroke. The LGI status, measured by a composite score, was also an independent predictor of recurrent stroke (HR, 1.103; 95% CI, 1.077–1.130; *P*<0.001). The association of LGI status with recurrent stroke using Kaplan–Meier analysis is shown in Figure 2.

Table 3 exhibits the associations of LGI score with stroke recurrence. In the multivariate Cox regression analysis, after adjusting for age and sex, patients with LGI score in the fourth quartile, compared with the first quartile, were associated with a higher risk of stroke recurrence (HR, 4.833; 95% CI, 2.994–7.799, *P*<0.001; Model 1). In model 2, after adjusting for age, sex, and confounders with a *P*-value <0.1 in univariate analysis, LGI score in the fourth quartile was independently associated with recurrent stroke (HR, 4.080; 95% CI, 2.420–6.879; *P*<0.001),

and the c-index of the final model was 0.722. The area under the ROC curve of the LGI score was highest compared with the single biomarker (Figure 3).

After adjusting for the potential confounders in the aforementioned model 2, multivariate competing-risk regression analysis confirmed that the LGI score was also an independent predictor of higher risk of recurrent stroke (The fourth quartile versus the first quartile; HR, 3.555; 95% CI, 1.802–7.012; *P*<0.001).

Subgroup Analysis

In a further sensitivity analysis, after controlling the possible confounders with stratified analysis, the LGI score in the fourth quartile was independently associated with the risk of stroke recurrence (Table 4). Furthermore, a higher risk of increasing LGI score was found in the participants with a lower level of education (*P* for interaction <0.001, Table 4), which suggested a significant interaction between LGI status and education levels.

Supplemental Table 1 shows the comparisons of LGI status in different stroke subtypes. Compared with patients without recurrence, the proportion of the fourth quartile of LGI score was significantly higher in recurrent ischemic stroke or intracranial hemorrhage patients (19.1% versus 39.0, 19.1% versus 55.6%, both *P*<0.05), while there was no significant difference between the ischemic stroke and intracranial hemorrhage groups (39.0% versus 55.6%, *P*>0.05).

Discussion

In the present study, we performed a score of LGI based on plasmatic (CRP) and cellular (WBC count, PLT count, and NLR) biomarkers in patients with first-ever ischemic stroke, and found that it was an independent predictor of stroke recurrence.

Recurrent stroke is more devastating with prolonged hospitalization and higher morbidity compared with the first-ever stroke.[24] LGI status was the common soil involving pathogenetic mechanism among several age-related chronic diseases,[7–9] but the association between increased LGI condition and stroke recurrence is not well established. Several studies have reported that the CRP, WBC count, PLT count, and NLR are reliable indicators of inflammation status.[11,12,16] The processes of inflammation and recurrent stroke are complex. Numerous studies have explored the association between LGI condition and stroke recurrence only using a single biomarker approach, rather than considering a panel of combined selected
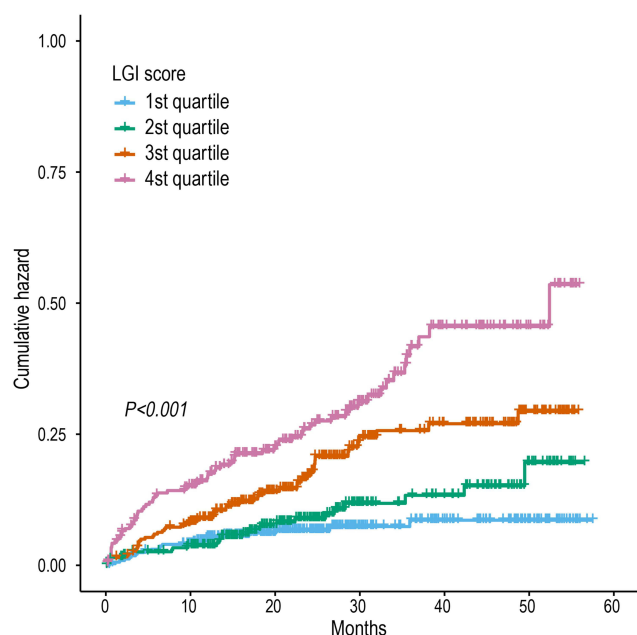

Figure 2 Cumulative incidence rates of stroke recurrence according to the quartile of low-grade inflammation score.
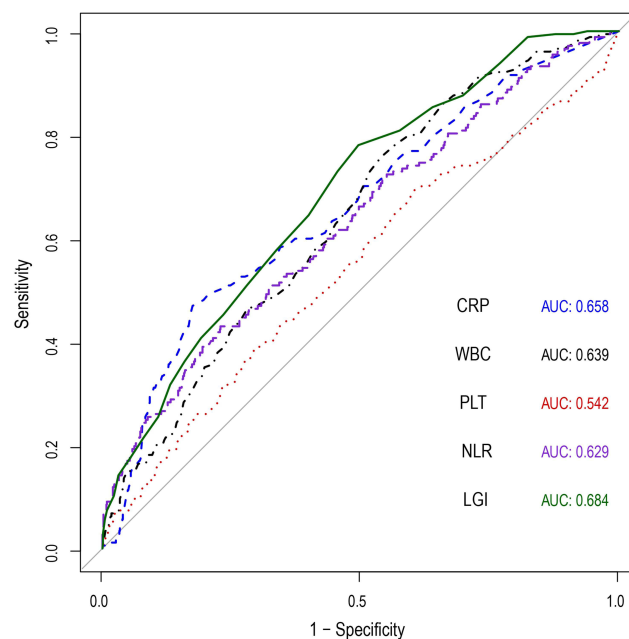**Abbreviation**: LGI, low-grade inflammation.

Table 3 Associations of Low-Grade Inflammation Score with Stroke Recurrence

Variables	Cox Regression Model		Competing Risk Analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Crude model				
Quartile of LGI score				
1st quartile	1.00 (ref)		1.00 (ref)	
2nd quartile	1.443 (0.818–2.546)	0.205	1.430 (0.811–2.530)	0.220
3rd quartile	2.687 (1.643–4.395)	<0.001	2.690 (1.640–4.400)	<0.001
4th quartile	4.312 (2.675–6.952)	<0.001	4.190 (2.597–6.770)	<0.001
Model 1				
Quartile of LGI score				
1st quartile	1.00 (ref)		1.00 (ref)	
2nd quartile	1.516 (0.859–2.677)	0.151	1.480 (0.842–2.610)	0.170
3rd quartile	2.745 (1.678–4.491)	<0.001	2.740 (1.675–4.480)	<0.001
4th quartile	4.833 (2.994–7.799)	<0.001	4.550 (2.815–7.350)	<0.001
Model 2				
Quartile of LGI score				
1st quartile	1.00 (ref)		1.00 (ref)	
2nd quartile	1.390 (0.787–2.455)	0.257	1.361 (0.752–2.462)	0.310
3rd quartile	2.376 (1.446–3.903)	0.001	2.286 (1.325–3.945)	0.003
4th quartile	4.080 (2.420–6.879)	<0.001	3.555 (1.802–7.012)	<0.001

Notes: Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, stroke subtype, education years, smoking status, CRP, WBC count, PLT count, and NLR.
Abbreviations: HR, Hazard ratio; CI, Confidence interval; CRP, C-reactive protein; WBC, White blood cell; PLT, Platelet; NLR, Neutrophil-to-lymphocyte ratio; LGI, low-grade inflammation.

biomarkers.[13–15,25] Thus we presented an LGI score to evaluate the comprehensive effects on recurrent stroke.[26]

Figure 3 The receiver operating characteristic curves of the low-grade inflammation score and the individual biomarkers.
Abbreviations: CRP, C-reactive protein; WBC, white blood cell; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; LGI, low-grade inflammation score.

The composite score used by Bonaccio et al[11] is a feasible scale to assess the relationship between LGI status and total mortality. In the present study, the fourth quartile of LGI score was significantly associated with the increasing risk of stroke recurrence both in the Cox proportional hazards models and the multivariate competing-risk analyses. Of notice, when we additionally adjusted for age, sex, and factors with a *P*-value <0.1 in the univariate analysis, the effect of LGI on recurrent stroke remained robust, which meant that the LGI status was the independent predictor of stroke recurrence. Furthermore, we also evaluated the specific contribution of each biomarker using the ROC curve: CRP, WBC count, and NLR made a significant contribution to the correlation between LGI score and stroke recurrence, nevertheless, the contribution of PLT also should not be neglected. Moreover, blood pressure (BP) at admission,[27] baseline stroke severity,[28] stroke etiology,[29] and education levels[11] were all related to increased LGI status, which was in line with our research. In the sensitivity analysis, we dichotomized the abovementioned LGI-related factors and test the interaction effect, and found that the association of LGI status with stroke recurrence was higher in the subjects with a lower education level. The possible explanations accounting for

Table 4 Sensitivity Analysis for the Associations Between Low-Grade Inflammation Score and Stroke Recurrence

Subgroups	N of Events/N of Subjects	Quartile of LGI Score (HR, 95% CI)				P for Interaction
		1st	2nd	3rd	4th	
Sex						0.060
Male	869/1214	1.0 (Ref)	1.465 (0.780–2.750)	2.130 (1.211–3.748)	3.137 (1.709–5.756)	
Female	342/1214	1.0 (Ref)	0.610 (0.138–2.710)	2.544 (0.895–7.232)	6.292 (2.208–17.931)	
Age						0.339
<65 years	766/1214	1.0 (Ref)	1.941 (0.813–4.637)	2.845 (1.291–6.265)	2.545 (1.088–5.953)	
≥65 years	447/1214	1.0 (Ref)	0.990 (0.451–2.174)	2.134 (1.082–4.208)	5.654 (2.598–12.307)	
Stroke subtype						0.608
LAA	469/1214	1.0 (Ref)	1.099 (0.445–2.719)	1.774 (0.814–3.866)	3.235 (1.448–7.227)	
CE	112/1214	1.0 (Ref)	9.755 (0.864–110.134)	7.307 (0.682–78.339)	21.707 (2.574–183.084)	
SAA	245/1214	1.0 (Ref)	2.086 (0.464–9.384)	4.743 (1.253–17.948)	11.191 (3.003–41.699)	
Others	384/1214	1.0 (Ref)	0.878 (0.331–2.327)	1.528 (0.656–3.555)	1.550 (0.504–4.770)	
Education						<0.001
0–6	314/1214	1.0 (Ref)	2.047 (0.489–8.569)	5.422 (1.589–18.493)	16.917 (4.907–58.328)	
6–9	618/1214	1.0 (Ref)	1.107 (0.497–2.468)	1.552 (0.757–3.182)	1.323 (0.534–3.274)	
9–12	143/1214	1.0 (Ref)	1.432 (0.303–6.763)	1.856 (0.456–7.556)	18.551 (3.320–103.643)	
>12	138/1214	1.0 (Ref)	1.538 (0.284–8.339)	1.943 (0.240–15.715)	1.905 (0.055–65.855)	
Smoker status						0.752
Nonsmokers	515/1214	1.0 (Ref)	1.032 (0.289–3.682)	3.801 (1.443–10.008)	9.899 (3.371–29.069)	
Former	115/1214	1.0 (Ref)	2.002 (0.449–8.926)	2.804 (0.573–13.723)	11.604 (1.183–113.873)	
Quitters	325/1214	1.0 (Ref)	0.675 (0.170–2.691)	0.989 (0.265–3.691)	1.082 (0.185–6.328)	
Persistent	254/1214	1.0 (Ref)	1.515 (0.562–4.088)	1.742 (0.645–4.705)	1.923 (0.581–6.357)	
Baseline NIHSS score						0.630
<5	703/1214	1.0 (Ref)	1.513 (0.740–3.093)	1.822 (0.909–3.652)	2.243 (0.861–5.843)	
≥5	510/1214	1.0 (Ref)	0.940 (0.325–2.720)	2.371 (1.010–5.563)	5.110 (2.262–11.547)	
Baseline SBP						0.326
<140 mmHg	541/1214	1.0 (Ref)	1.562 (0.662–3.689)	2.995 (1.451–6.184)	3.502 (1.639–7.482)	
≥140 mmHg	671/1214	1.0 (Ref)	1.157 (0.536–2.498)	1.843 (0.935–3.633)	3.947 (1.961–7.946)	
Baseline DBP						0.619
<90 mmHg	873/1214	1.0 (Ref)	1.334 (0.711–2.504)	2.516 (1.470–4.308)	3.664 (2.127–6.312)	
≥90 mmHg	339/1214	1.0 (Ref)	2.415 (0.591–9.875)	3.334 (0.921–12.076)	8.441 (2.313–30.806)	

Notes: HR and 95% CI were calculated after adjustment for the same variables as model 2 in Table 3, except for the stratified variable.
Abbreviations: LGI, low-grade inflammation; HR, hazard ratio; CI, confidence interval; LAA, large-artery atherosclerosis; CE, cardioembolism; SAA, small-vessel occlusion; NIHSS, National Institute of Health Stroke Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure.

this phenomenon are as follows: patients with higher educational levels are more able to control their lifestyle and obey prescriptions after stroke,[30–32] which are helpful to reduce inflammation and prevent the probability of recurrence.

The possible mechanisms of increased LGI condition on the stroke recurrence are not well defined. CRP has several potential anti-inflammation properties and proinflammatory effects, which may contribute to the progress of atherothrombosis and ischemic injury.[33,34] Activation of circulating WBC might disturb the microvascular flow and activate the PLT that is associated with endothelial dysfunction and the rupture of the vulnerable plaque.[26] NLR

is considered a good comprehensive indicator of acute inflammation as well as a predictor of recurrent stroke.[35] Thus, chronic LGI status may be the pivotal factor of atherogenesis, which may ultimately lead to atherosclerotic plaque rupture and induce stroke recurrence. The LGI score, effectively controlling the variability of inflammation biomarkers and considering their possible synergistic effect, was a good predictor of recurrent stroke.

To our knowledge, this is the first study to explore the risk of recurrent stroke in association with a clinically silent LGI status measured by a composite score. The major strengths of this study include a large hospital-

based cohort, comprehensive assessment of LGI status, and a quite long follow-up period, all of which make this group appropriate for investigating the association between LGI and recurrent stroke. Of notice, to avoid overestimating the risk of LGI status in stroke recurrence, we excluded the patients with acute inflammation or any kind of hematological disease. However, this study has several limitations that should be noted when interpreting the results. First, patients with first-ever ischemic stroke registering in the prospective NSRP may not be generalized to other populations. Second, lifestyle modifications, better patient care, and compliance with medical treatment may interfere with our findings even we considered numerous confounders. Third, we only measured the baseline level of LGI markers and the effect of fluctuation of LGI could not be eliminated. Also, because of the unavailability of the data, other inflammation indicators like tumor necrosis factor-alpha, interleukin-6, and interleukin-8 were not included in the LGI score. Further studies are warranted to assess whether or not the chronic reduction in LGI could decrease the risk of recurrent stroke.

Conclusion

The present study showed that increased LGI score concerning all possible synergic effects of LGI biomarkers could independently predict stroke recurrence.

Data Sharing and Data Accessibility

The data in this study are available from the corresponding author (Xinfeng Liu) on a reasonable request.

Ethics Approval and Informed Consent

Investigation on the NSRP was approved by the Ethics Review Board of Jinling Hospital (approval number 2010NLY-018).

Author Contributions

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

Disclosure

All authors report no conflicts of interest in this work.

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