

# Serum YKL-40 Levels and White Matter Hyperintensities in Patients with Acute Ischemic Stroke

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**Background:** White matter hyperintensity (WMH) is associated with risk of acute ischemic stroke (AIS) and poor outcomes after AIS. The purpose of this prospective study was to evaluate the association between serum YKL-40 levels and WMH burden in patients with AIS.

**Methods:** From February 2020 to March 2021, a total of 672 consecutive AIS patients with magnetic resonance imaging data were prospectively recruited from two centers. Serum YKL-40 levels were quantified using enzyme-linked immunosorbent assay. The burden of WMH was semiquantitatively measured by the Fazekas visual grading scale. According to severity of overall WMH, patients were dichotomized into none–mild WMH group (Fazekas score 0–2) or moderate–severe WMH group (Fazekas score 3–6). Besides, based on severity of periventricular WMH (PV-WMH) and deep WMH (D-WMH), patients were categorized as none–mild (Fazekas score 0–1) or moderate–severe (Fazekas score 2–3).

**Results:** Among the 672 patients, 335 (49.9%) participants were identified with moderate–severe overall WMH, 326 (48.5%) with moderate–severe PV-WMH and 262 (39.0%) with moderate–severe D-WMH. Compared with the first quartile of serum YKL-40, the adjusted odds ratio (OR) of the fourth quartile for moderate–severe PV-WMH was 2.473 (95% confidence interval [CI] 1.316–4.646;  $P=0.005$ ). No significant association was observed between YKL-40 and overall WMH (OR 0.762; 95% CI 0.434–1.336;  $P=0.343$ ) or D-WMH (OR 0.695; 95% CI 0.413–1.171;  $P=0.172$ ).

**Conclusion:** Our results suggested that higher YKL-40 levels appeared to be associated with PV-WMH, but not with overall WMH or D-WMH in patients with AIS.

**Keywords:** acute ischemic stroke, YKL-40, white matter hyperintensities, biomarkers

## Background

Stroke is reported to be one of the leading causes of disability and death around the world, especially in China.<sup>1</sup> White matter hyperintensity (WMH), a standout neuroimaging feature of chronic cerebral small vessel disease (CSVD),<sup>2</sup> is associated with an increased risk of ischemic stroke, poor clinical prognosis after stroke, as well as recurrent stroke.<sup>3,4</sup> To date, the underlying pathophysiology of WMH is poorly understood. Although age and traditional vascular risk factors are widely regarded as the main risk factors for WMH,<sup>2,5</sup> they are not responsible for all the pathogenesis and progression of WMH. Therefore, identifying novel related risk factors is crucial and would improve current understanding of the etiology and pathogenesis of WMH in patients with acute ischemic stroke (AIS).

Neuroinflammatory process is getting more and more attention for its pivotal role in the progression of both acute and chronic cerebrovascular disease.<sup>6,7</sup> Several inflammatory biomarkers have been identified as candidate risk factors for WMH, such as vascular cell adhesion molecule-1, lipoprotein-associated phospholipase A2, and so on.<sup>8,9</sup> YKL-40, also called chitinase-3-like-1 protein (CHI3L1) or breast regression protein 39 (BRP-39), is a novel biomarker of inflammation and plays a crucial role in angiogenesis, tissue fibrosis, inflammation, oxidative tissue injury, and extracellular remodeling responses.<sup>10,11</sup> Elevated YKL-40 levels have been reported to be associated with atherosclerosis,<sup>12</sup> cardiovascular disease<sup>13</sup> and cerebrovascular disease.<sup>14</sup> Recently, the cerebrospinal fluid (CSF) YKL-40 levels had been recognized as a pathophysiological biomarker for neurological diseases, such as Alzheimer's disease, Huntington's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis.<sup>15,16</sup> Nevertheless, the relationship between serum YKL-40 and WMH burden in AIS patients remained unknown and was waiting for neurologists to explore.

We hypothesized that increased serum YKL-40 levels may be linked to severity of WMH. Based on this hypothesis, a prospective cross-sectional study was conducted to assess the correlation between serum YKL-40 levels at admission and severity of WMH in patients with AIS. Additionally, we explored the associations of YKL-40 with WMH burden in different regions.

## Materials and Methods

### Study Population

Between February 2020 and March 2021, a total of 672 AIS patients admitted to Stroke Center of the Taixing People's Hospital and Nanjing First Hospital were consecutively recruited. The Ethics Committees of the two participating centers reviewed and approved the study. Informed consent was acquired from each participant/representative. This study was also complied with the Declaration of Helsinki.

### Inclusion and Exclusion Criteria

Patients were enrolled if they (1) aged 18 years or older, (2) were admitted within 48 hours of symptom onset, (3) underwent brain magnetic resonance imaging (MRI) including T2 fluid-attenuated inversion recovery (FLAIR) sequence. We excluded patients with (1) bilateral cerebral hemisphere infarction, (2) history of brain operation or trauma, (3) abnormalities on brain MRI (eg, neoplasm, hydrocephalus, or autoimmune encephalitis).

### Data Collection

For each participant, we collected demographic characteristics and past medical history including age, gender, hypertension, diabetes mellitus, lipid metabolism disorders, atrial fibrillation, coronary heart disease, previous stroke or transient ischemic attack (TIA), smoking, and drinking. Besides, we recorded each participant's laboratory data including levels of leucocyte, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), and hyper-sensitive C-reactive protein (hs-CRP). Clinical assessment (blood pressure, previous antiplatelet, previous statin, and stroke etiology) of each AIS patient was also collected. Stroke etiology was classified in light of Trial of ORG 10,172 in Acute Stroke Treatment (TOAST) criteria.<sup>17</sup>

### Measurement of YKL-40

Blood samples were collected within the first 24 hours of admission after overnight fasting. Serum samples were centrifuged (1500 rpm, 4°C, 15 min) and frozen at -80°C until later analysis. Serum concentrations of YKL-40 were determined with a four-parameter curve using a commercial enzyme-linked immunosorbent assay kit (Cat No. ab255719, Abcam). Intra-panel calibration was performed in line with the manufacturer's instructions, where the calculated minimal detectable dose is 3.9 pg/mL. The mean intra-assay and inter-assay coefficients of variation for YKL-40 were shown to be 2.4% and 1.7%, respectively.

## Imaging Assessment

Brain MRI was performed with two different 3.0 Tesla scanners (Verio Dot, Siemens, Erlangen, Germany and Ingenia, Philips, Best, Netherlands). Detailed MRI acquisition included T1-weighted sequence, T2-weighted sequence and T2 FLAIR sequence. WMH can be localised to two anatomically distinct regions: the area adjacent to the ventricles (periventricular WMH, PV-WMH) and the area under the cortex (deep WMH, D-WMH).<sup>18</sup> Both PV-WMH and D-WMH were semiquantitatively measured at T2-weighted as well as FLAIR sequences using the Fazekas visual grading scale,<sup>19</sup> which ranges from 0 to 3, by two experienced neuroradiologists who were blinded to all clinical data. We categorized the severity of PV-WMH and D-WMH as none–mild (Fazekas score 0–1) or moderate–severe (Fazekas score 2–3). The sum of PV-WMH and D-WMH scores was used to characterize the severity of overall WMH. All participants were dichotomized into none–mild (Fazekas score 0–2) and moderate–severe (Fazekas score 3–6) groups according to severity of overall WMH.

## Statistical Analysis

Statistical analyses were conducted by SPSS (version 26; SPSS Inc, Chicago, IL, USA). Continuous variables of an abnormal distribution were presented as the medians (interquartile range [IQR]), and categorical variables were presented as numbers (percentages [%]). Continuous variables were compared using analysis of variance, Kruskal–Wallis *H*-test, Student's *t*-test or Mann–Whitney *U*-test, as appropriate. Categorical variables were compared using Pearson's chi-square test or Fisher exact test, as appropriate. Spearman rank correlation was used to identify the association between serum YKL-40 levels and Fazekas scores. Univariate and multivariate logistic regression analyses were performed to explore the relationship between serum YKL-40 levels and the severity of overall WMH, PV-WMH as well as D-WMH. Adjustments were made for age, gender, and the variables with  $P < 0.1$  in univariate analyses. All tests were 2-tailed, and statistical significance threshold was set at  $P < 0.05$ .

## Results

### Baseline Clinical Characteristics

A total of 672 AIS patients were consecutively recruited from February 2020 to March 2021 in our study. Among the included patients, the median age was 70 years, and 419 (62.4%) patients were male. The median level of serum YKL-40 at admission was 134.26 ng/mL. There were 337 (50.1%) patients with none–mild overall WMH (Fazekas score 0–2) and 335 (49.9%) patients with moderate–severe overall WMH (Fazekas score 3–6). Baseline clinical characteristics of participants by the degree of overall WMH are shown in Table 1. When comparing patients with none–mild overall WMH, those with moderate–severe overall WMH tended to be older ( $P=0.001$ ) and had higher levels of FBG ( $P=0.014$ ), higher proportions of hypertension ( $P=0.001$ ), previous stroke or TIA ( $P=0.006$ ) and subtype of large-artery atherosclerosis ( $P=0.002$ ); while the levels of serum YKL-40 ( $P=0.754$ ) were not significantly different.

### Associations of YKL-40 with Overall WMH, PV-WMH, and D-WMH

Table 2 demonstrates Fazekas scores among patients according to quartiles of YKL-40. Compared with the lowest quartile of serum YKL-40, patients in the fourth quartile had higher Fazekas scores of overall WMH ( $P=0.029$ ) and PV-WMH ( $P=0.001$ ). However, there was no significant difference in D-WMH among the four groups ( $P=0.973$ ).

We further assessed the associations of PV-WMH and D-WMH with serum YKL-40 levels separately. There were 346 (51.5%) patients with none–mild PV-WMH and 326 (48.5%) patients with moderate–severe PV-WMH. Compared with patients in none–mild PV-WMH group, those in moderate–severe PV-WMH group were older and had a higher prevalence of hypertension, previous stroke or TIA and subtype of large-artery atherosclerosis; lower levels of TG; and higher levels of FBG, hs-CRP and YKL-40 (Table 3). When grouped according to severity of D-WMH, 410 (61.0%) and 262 (39.0%) patients were in the none–mild and moderate–severe D-WMH groups, respectively. Patients with moderate–severe D-WMH were older and had higher proportion of hypertension, previous stroke or TIA, smoking and subtype of large-artery atherosclerosis; and higher levels of FBG (Table 3).

**Table 1** Baseline Characteristics of Participants by the Degree of Overall WMH

Variables	All Patients (n = 672)	Overall WMH		P
		None-Mild (n = 337)	Moderate-Severe (n = 335)	
Demographic characteristics				
Age, years	70 (61, 78)	62 (55, 70)	77 (70, 82)	0.001
Male, n (%)	419 (62.4)	214 (63.5)	205 (61.2)	0.537
Past medical history, n (%)				
Hypertension	465 (69.2)	197 (58.5)	268 (80.0)	0.001
Diabetes mellitus	152 (22.6)	79 (23.4)	73 (21.8)	0.609
Lipid metabolism disorders	91 (13.5)	41 (12.2)	50 (14.9)	0.296
Atrial fibrillation	115 (17.1)	51 (15.1)	64 (19.1)	0.172
Coronary artery disease	104 (15.5)	48 (14.2)	56 (16.7)	0.375
Previous stroke or TIA	121 (18.0)	47 (13.9)	74 (22.1)	0.006
Smoking	288 (42.9)	136 (40.4)	152 (45.4)	0.189
Drinking	226 (33.6)	119 (35.3)	107 (31.9)	0.355
Clinical assessment				
Systolic blood pressure (mmHg)	150 (136, 163)	146 (130, 165)	150 (139, 164)	0.121
Diastolic blood pressure (mmHg)	87 (80, 96)	87 (80, 98)	87 (79, 96)	0.350
Previous antiplatelet, n (%)	95 (14.1)	45 (13.4)	50 (14.9)	0.559
Previous statin, n (%)	78 (11.6)	39 (11.6)	39 (11.6)	0.978
TOAST subtype				
LAA	306 (45.5)	147 (43.6)	159 (47.5)	0.002
CE	130 (19.3)	54 (16.0)	76 (22.7)	
SAO	203 (30.2)	115 (34.1)	88 (26.3)	
SOE	9 (1.3)	9 (2.7)	0 (0.0)	
SUE	24 (3.6)	12 (3.6)	12 (3.6)	
Laboratory data				
Leukocyte ( $\times 10^9/L$ )	7.54 (6.14, 9.42)	7.68 (6.14, 9.53)	7.44 (6.16, 9.11)	0.393
TC (mmol/L)	4.64 (3.96, 5.25)	4.70 (4.02, 5.21)	4.58 (3.93, 5.26)	0.463
TG (mmol/L)	1.28 (0.91, 1.78)	1.27 (0.97, 1.85)	1.33 (0.88, 1.74)	0.264
HDL-C (mmol/L)	1.16 (0.99, 1.34)	1.15 (0.96, 1.34)	1.17 (1.01, 1.34)	0.288
LDL-C (mmol/L)	2.52 (2.03, 3.15)	2.46 (2.04, 3.18)	2.57 (2.01, 3.14)	0.992
FBG (mmol/L)	5.80 (5.01, 7.23)	5.67 (4.89, 7.19)	5.90 (5.18, 7.26)	0.014
hs-CRP (mg/L)	3.73 (1.47, 8.51)	3.56 (1.51, 8.62)	3.98 (1.40, 8.52)	0.269
YKL-40 (ng/mL)	134.26 (76.56, 240.17)	134.19 (73.55, 238.42)	135.73 (77.24, 242.30)	0.754

**Notes:** Continuous variables are expressed as medians (IQR), and categorical variables are presented as n (%).

**Abbreviations:** WMH, white matter hyperintensity; TIA, transient ischemic attack, TOAST, Trial of ORG 10,172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; CE, cardioembolism; SAO, small-artery occlusion; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; hs-CRP, hyper-sensitive C-reactive protein.

**Table 2** Fazekas Scores Among Patients According to Quartiles of YKL-40

Fazekas Score	Serum YKL-40 Levels, ng/mL				P
	First Quartile (23.63–76.51)	Second Quartile (76.56–134.26)	Third Quartile (134.27–239.82)	Fourth Quartile (240.17–798.14)	
Overall WMH	2 (1, 4)	3 (1, 4)	2 (1, 4)	3 (2, 4)	0.029
PV-WMH	1 (1, 2)	1 (1, 2)	1 (1, 2)	1 (2, 2)	0.001
D-WMH	1 (0, 2)	1 (1, 2)	1 (0, 2)	1 (0, 2)	0.973

**Notes:** Fazekas scores are expressed as medians (IQR).

**Abbreviations:** WMH, white matter hyperintensity; PV-WMH, periventricular white matter hyperintensity; D-WMH, deep white matter hyperintensity.

Levels of YKL-40 in the overall WMH, PV-WMH, and D-WMH groups are depicted in [Figure 1](#). Serum YKL-40 levels of AIS patients were higher in moderate-severe PV-WMH group than those in none-mild PV-WMH group (150.21 [81.13, 261.60] versus 130.94 [63.53, 232.33] ng/mL,  $P=0.007$ ). Furthermore, when comparing with first quartile of serum YKL-40, the adjusted odds ratio (OR) of the fourth quartile for moderate-severe PV-WMH was

**Table 3** Characteristics of Participants the Degree of PV-WMH and D-WMH

Variables	PV-WMH		P	D-WMH		P
	None-Mild (n = 346)	Moderate-Severe (n = 326)		None-Mild (n = 410)	Moderate-Severe (n = 262)	
Demographic characteristics						
Age, years	62 (55, 69)	77 (71, 83)	0.001	66 (57, 73)	77 (69, 83)	0.001
Male, n (%)	219 (63.3)	200 (61.3)	0.603	251 (61.2)	168 (64.1)	0.449
Past medical history, n (%)						
Hypertension	201 (58.1)	264 (81.0)	0.001	260 (63.4)	203 (78.2)	0.001
Diabetes mellitus	80 (23.1)	72 (22.1)	0.748	94 (22.9)	58 (22.1)	0.811
Lipid metabolism disorders	49 (14.2)	42 (12.9)	0.628	56 (13.7)	35 (13.4)	0.912
Atrial fibrillation	53 (15.3)	62 (19.0)	0.203	63 (15.4)	52 (19.8)	0.132
Coronary artery disease	47 (13.6)	57 (17.5)	0.162	60 (14.6)	44 (16.8)	0.450
Previous stroke or TIA	44 (12.7)	77 (23.6)	0.001	62 (15.1)	59 (22.5)	0.015
Smoking	144 (41.6)	144 (44.2)	0.504	163 (39.8)	125 (47.7)	0.042
Drinking	128 (37.0)	98 (30.1)	0.057	136 (33.2)	90 (34.4)	0.752
Clinical assessment						
Systolic blood pressure (mmHg)	150 (134, 162)	147 (137, 164)	0.757	150 (135, 164)	148 (137, 163)	0.594
Diastolic blood pressure (mmHg)	87 (80, 99)	88 (79, 96)	0.273	87 (80, 96)	88 (79, 96)	0.807
Previous antiplatelet, n (%)	42 (12.1)	53 (16.3)	0.126	59 (14.4)	36 (13.7)	0.814
Previous statin, n (%)	37 (10.7)	41 (12.6)	0.446	50 (12.2)	28 (10.7)	0.552
TOAST subtype			0.001			0.042
LAA	145 (41.9)	161 (49.4)		180 (43.9)	126 (48.1)	
CE	54 (15.6)	76 (23.3)		74 (18.0)	56 (21.4)	
SAO	125 (36.1)	78 (23.9)		134 (32.7)	69 (26.3)	
SOE	8 (2.3)	1 (0.3)		9 (2.2)	0 (0.0)	
SUE	14 (4.0)	10 (3.1)		13 (3.2)	11 (4.2)	
Laboratory data						
Leukocyte ( $\times 10^9/L$ )	7.52 (6.05, 9.32)	7.56 (6.42, 9.61)	0.243	7.55 (6.17, 9.45)	7.51 (6.09, 9.39)	0.967
TC (mmol/L)	4.70 (4.04, 5.21)	4.57 (3.91, 5.28)	0.259	4.69 (4.03, 5.21)	4.53 (3.90, 5.31)	0.364
TG (mmol/L)	1.28 (0.97, 1.88)	1.28 (0.86, 1.74)	0.037	1.23 (0.93, 1.79)	1.36 (0.88, 1.77)	0.945
HDL-C (mmol/L)	1.16 (0.95, 1.34)	1.17 (1.02, 1.34)	0.228	1.16 (0.99, 1.34)	1.16 (1.00, 1.34)	0.637
LDL-C (mmol/L)	2.48 (2.00, 3.16)	2.57 (2.04, 3.14)	0.857	2.50 (2.03, 3.19)	2.57 (2.03, 3.11)	0.909
FBG (mmol/L)	5.64 (4.89, 7.17)	6.07 (5.21, 7.33)	0.004	5.70 (4.95, 7.26)	5.91 (5.19, 7.20)	0.025
hs-CRP (mg/L)	3.49 (1.49, 8.12)	4.28 (1.40, 9.02)	0.033	3.57 (1.51, 8.49)	3.98 (1.33, 8.55)	0.265
YKL-40 (ng/mL)	130.94 (63.53, 232.33)	150.21 (81.13, 261.60)	0.007	134.61 (78.49, 239.94)	130.83 (76.26, 241.12)	0.228

**Notes:** Continuous variables are expressed as medians (IQR), and categorical variables are presented as n (%).

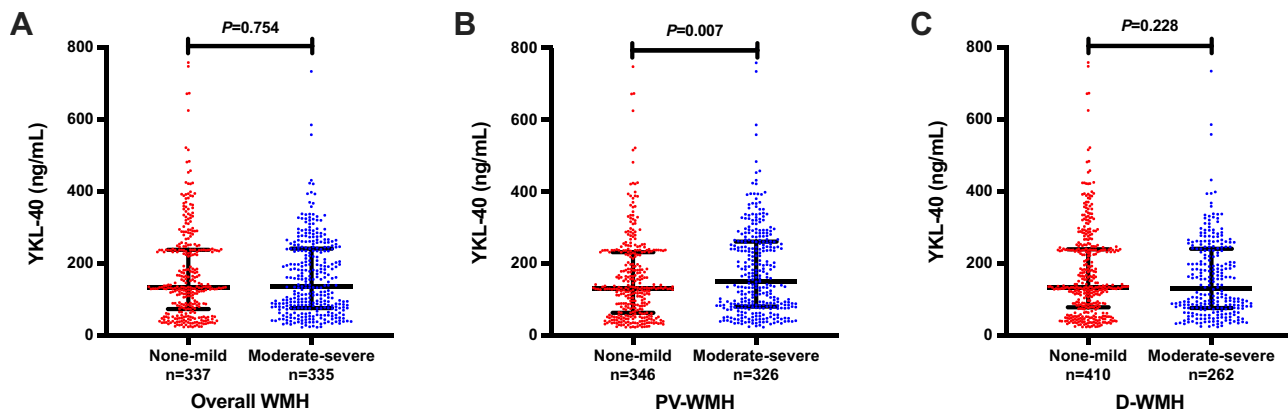
**Abbreviations:** PV-WMH, periventricular white matter hyperintensity; D-WMH, deep white matter hyperintensity; TIA, transient ischemic attack, TOAST, Trial of ORG 10,172 in Acute Stroke Treatment; LAA, large-artery atherosclerosis; CE, cardioembolism; SAO, small-artery occlusion; SOE, stroke of other determined etiology; SUE, stroke of undetermined etiology; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; hs-CRP, hyper-sensitive C-reactive protein.

2.473 (95% confidence interval [CI] 1.316–4.646;  $P=0.005$ ) after adjusting for age, gender, and variables with  $P < 0.1$  in univariate analysis. However, there was no significant association between YKL-40 with overall WMH or D-WMH (Table 4).

## Discussion

In this prospective cross-sectional study, we demonstrated that baseline serum YKL-40 levels were statistically associated with severity of PV-WMH in patients with AIS. This relationship was independent of age, gender, and other potential confounding variables. However, no significant associations were found between YKL-40 with overall WMH or D-WMH.

Our study showed a prevalence of 49.9% for moderate–severe overall WMH, 48.5% for moderate–severe PV-WMH and 39.0% for moderate–severe D-WMH, which were in parallel with previous researches,<sup>20,21</sup> while higher than findings (36.2% for moderate–severe PV-WMH and 15.0% for moderate–severe D-WMH) by Zong and colleagues.<sup>22</sup> Differences in age distributions of the participants may be the main reason for this discrepancy. The mean age was  $62.3 \pm 11.5$  years in the



**Figure 1** Serum YKL-40 levels in different groups according to (A) overall WMH, (B) PV-WMH and (C) D-WMH in AIS patients. Horizontal lines represent medians (IQR). **Abbreviations:** WMH, white matter hyperintensity; PV-WMH, periventricular white matter hyperintensity; D-WMH, deep white matter hyperintensity.

study by Zong and colleagues,<sup>22</sup> which was lower than that in our study ( $69.0 \pm 12.0$  years). Previous studies have demonstrated that increasing age was the most prominent risk factor for WMH,<sup>23,24</sup> and our results also confirmed this. In addition, our present study revealed that both PV-WMH and D-WMH were significantly associated with hypertension, previous stroke or TIA, as well as higher blood glucose levels, which were consistent with previous results.<sup>25</sup>

The precise pathophysiological mechanisms of WMH have not been fully elucidated, but may include hypoxia-ischemia, blood-brain barrier (BBB) breakdown, endothelial dysfunction, oxidative stress, venous collagen deposition.<sup>26,27</sup> In recent years, increasing evidence indicates an extraordinarily critical role of inflammation in the progression of WMH.<sup>9,24,28,29</sup> As a glycoprotein mediating inflammation, YKL-40 has been reported to correlated with pulmonary diseases, diabetes mellitus, coronary heart disease, Alzheimer’s disease and stroke.<sup>10,11,14</sup> However, evidence for the correlation between YKL-40 and CSVD is limited. In a mouse model of WMH induced by chronic cerebral

**Table 4** Logistic Regression Analyses of YKL-40 with White Matter Hyperintensity

YKL-40	Overall WMH		PV-WMH		D-WMH	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Unadjusted model						
First quartile	Reference		Reference		Reference	
Second quartile	1.154 (0.752–1.770)	0.513	1.273 (0.827–1.958)	0.273	0.975 (0.630–1.510)	0.911
Third quartile	1.024 (0.667–1.571)	0.913	0.976 (0.632–1.506)	0.912	0.904 (0.583–1.403)	0.654
Fourth quartile	1.210 (0.789–1.857)	0.383	2.456 (1.583–3.810)	0.001	0.975 (0.630–1.510)	0.911
Age- and sex-adjusted						
First quartile	Reference		Reference		Reference	
Second quartile	1.150 (0.673–1.964)	0.610	1.385 (0.786–2.440)	0.260	0.933 (0.565–1.540)	0.785
Third quartile	1.217 (0.714–2.075)	0.469	1.189 (0.678–2.086)	0.545	1.025 (0.620–1.695)	0.924
Fourth quartile	0.790 (0.458–1.362)	0.396	2.595 (1.434–4.697)	0.002	0.699 (0.421–1.162)	0.167
Multivariable adjusted*						
First quartile	Reference		Reference		Reference	
Second quartile	1.124 (0.649–1.945)	0.677	1.318 (0.733–2.369)	0.356	0.897 (0.539–1.493)	0.676
Third quartile	1.171 (0.679–2.021)	0.570	1.107 (0.614–1.995)	0.736	1.052 (0.631–1.752)	0.847
Fourth quartile	0.762 (0.434–1.336)	0.343	2.473 (1.316–4.646)	0.005	0.695 (0.413–1.171)	0.172

**Notes:** \*Overall WMH: adjusted for age, gender, hypertension, previous stroke, TOAST subtype, and FBG. PV-WMH: adjusted for age, gender, hypertension, previous stroke, drinking, TOAST subtype, TG, FBG, and Hs-CRP. D-WMH: adjusted for age, gender, hypertension, previous stroke or TIA, smoking, TOAST subtype, and FBG.

**Abbreviations:** WMH, white matter hyperintensity; PV-WMH, periventricular white matter hyperintensity; D-WMH, deep white matter hyperintensity; OR, odds ratio; CI, confidence interval.

hypoperfusion, the expression of YKL-40 was shown to be elevated.<sup>30</sup> A recently published community-based study with 960 Chinese stroke-free participants observed a significant association between elevated levels of endothelial-related inflammatory biomarkers including YKL-40 and increased WMH volume.<sup>9</sup> In another study with 42 sporadic CSVD patients, CSF levels of YKL-40 were positively correlated with the WMH load.<sup>31</sup> The results of our study suggested that serum YKL-40 levels at baseline, in AIS patients remained significantly associated with PV-WMH after adjusting for potential confounding risk factors, which has never been reported to our knowledge.

Although the exact mechanisms linking elevated YKL-40 and PV-WMH are still unclear, some possible explanations have been proposed. First of all, evidence from both Danish general population and Chinese stroke patients have shown that YKL-40 levels are positively correlated with increasing age,<sup>32,33</sup> and age is recognized as a prominent risk factor for PV-WMH,<sup>23,24</sup> which could partly explain the association between YKL-40 and PV-WMH. Secondly, overexpression of YKL-40 has been shown to up-regulate matrix metalloproteinase-9,<sup>34</sup> which is best known as a major contributor to degrade extracellular matrix and disrupt BBB permeability,<sup>35,36</sup> and further participate in the progression of WMH. Thirdly, as an endothelial-related inflammatory mediator, YKL-40 could activate endothelial cells to induce intercellular adhesion molecule-1 expression,<sup>37</sup> which would further recruit leukocytes and release pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$  and interferon- $\gamma$ , leading to endothelial dysfunction and BBB disruption.<sup>38,39</sup> Additionally, according to the latest research,<sup>30</sup> YKL-40 expression in astrocytes was elevated in white matter lesions caused by chronic hypoxia, and downregulating the expression of YKL-40 significantly alleviate white matter injury, suggesting that YKL-40 is implicated in the process of white matter injury. Taken together, the mechanisms mentioned above might be significant contributors to YKL-40 with PV-WMH.

Whereas our study suggested that elevated serum YKL-40 levels were associated with PV-WMH, no significant association was found between YKL-40 and D-WMH. To date, only a few studies investigated the differences between PV-WMH and D-WMH. In a prospective cross-sectional study involving 595 AIS patients, Yu et al showed that plasma phenylacetylglutamine levels in the fourth quartile was significantly associated with moderate-severe overall WMH (95% CI 1.134–4.018;  $P=0.019$ ) and PV-WMH (95% CI 1.174–4.226;  $P=0.014$ ), but not D-WMH (95% CI 0.981–3.372;  $P=0.057$ ).<sup>21</sup> Similarly, another recent study which detected the relationships of trimethylamine N-oxide with PV-WMH and D-WMH in a large cohort of TIA/AIS patients found that elevated trimethylamine N-oxide levels were associated with a higher risk of WMH burden, and more closely associated with PV-WMH than D-WMH.<sup>40</sup> We speculate that the discrepancy may be attributed to anatomical, histological as well as pathophysiological differences between PV-WMH and D-WMH. Previous histopathological studies have demonstrated that PV-WMH contained more immunoreactive microglia and astrocytes than D-WMH.<sup>41,42</sup> Pathology studies have also shown that PV-WMH is more likely related to inflammation and chronic hypoperfusion,<sup>43</sup> while D-WMH is more linked to ischemic damage.<sup>44</sup> Our data provided clinical evidence for the different pathological processes underlying PV-WMH and D-WMH, and further investigations about the detailed mechanisms are required.

Several potential limitations of our study should be mentioned. First, the samples were collected from AIS patients, thereby our results may not be able to reflect the general population. Second, due to the cross-sectional observational nature of this study, we cannot establish a causal relationship between YKL-40 and WMH. Third, as reported by a previous study, YKL-40 levels of AIS patients increased on the first day and peaked on the second day after admission.<sup>14</sup> However, our study only monitored YKL-40 levels at baseline and did not examine the dynamic changes of YKL-40, which may have provided more valuable information about the mechanism underlying the association between YKL-40 and WMH burden in AIS patients. Additionally, although the Fazekas visual rating scale is widely used to assess WMH burden, it is not as precise as quantitative evaluation. Therefore, our results should be interpreted with caution. Whereas YKL-40 was associated with PV-WMH severity in our study, it is not a risk factor for PV-WMH.

## Conclusions

Despite its inherent shortcomings, our data suggest that in AIS patients, elevated serum YKL-40 levels appear to be associated with PV-WMH severity, but not with D-WMH severity. Future studies are needed to validate the relationship and to further explore the exact mechanism linking YKL-40 and WMH.

## Data Sharing Statement

The relevant data supporting the conclusions of this study are available on reasonable request to Rujuan Zhou.

## Ethics Approval and Consent to Participate

The protocol was reviewed and approved by the Ethical Committee of the Taixing People's Hospital and Nanjing First Hospital. All participants or their legal representatives provided informed consent. The protocol was also conducted in accordance with the Declaration of Helsinki.

## Author Contributions

All authors made significant contributions 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.

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## Disclosure

All the authors declare that there is no conflict of interest for this work.

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