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Association of Uric Acid, High-Sensitivity C-Reactive Protein, and 90-Day Risk of Poor Function Outcome in Patients with Ischemic Stroke or Transient Ischemic Attacks

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Aim: The interaction between inflammatory biomarkers (high-sensitivity C-reactive protein, hsCRP) and antioxidants (uric acid, UA) regarding prognosis after ischemic stroke or transient ischemic attack (TIA) remains inadequately explored. This study aimed to assess (1) the individual and joint effects of hsCRP and UA, and (2) the neuroprotective role of UA in patients with elevated hsCRP levels concerning poor functional outcomes at 90 days.

Methods: A prospective cohort study was conducted involving 2140 consecutive ischemic stroke or TIA patients with hsCRP and UA levels. The primary outcome was defined as a poor functional outcome, indicated by a modified Rankin Scale (mRS) score of 3–6 at 90 days, with a shift in the mRS score as a secondary outcome. Logistic regression and propensity score (PS) analyses were employed to ensure robustness.

Results: Poor functional outcome occurred in 345 (16.1%) patients. Individual effects found that the highest quartiles of hsCRP (adjusted OR = 3.090; 95% CI 2.150–4.442) and UA (adjusted OR = 0.671; 95% CI 0.551–0.883) were associated with increased or decreased risk of poor functional outcome, respectively. Joint effects (adjusted OR = 3.994; 95% CI 2.758–5.640) between hsCRP and UA on the primary outcome were more apparent in patients with high hsCRP levels (hsCRP > 1.60 mg/L) and low UA levels (UA ≤ 291.85 μmol/L). For the patients with high hsCRP levels, patients with low UA levels had a higher risk of primary and secondary outcomes, compared with those with high UA levels, after unadjusted or adjusted for hsCRP. Similar and reliable results were observed in PS-based models.

Conclusion: In patients with ischemic stroke or TIA, joint high levels of hsCRP and low UA levels significantly correlate with increased risk of poor functional outcome at 90 days. In addition, high UA levels could reduce the risk of poor functional outcome for patients with high hsCRP levels.

Keywords: biomarkers, ischemic stroke, poor functional outcome, joint effects, neuroprotective effects

Introduction

Among neurological disorders, stroke is the leading cause of death and disability, with approximately 10 million individuals becoming permanently disabled and dying each year.[1] Ischemic stroke represents the most prevalent form of stroke, accounting for approximately 71% of all cases worldwide.[2] The consequences of ischemic stroke can be

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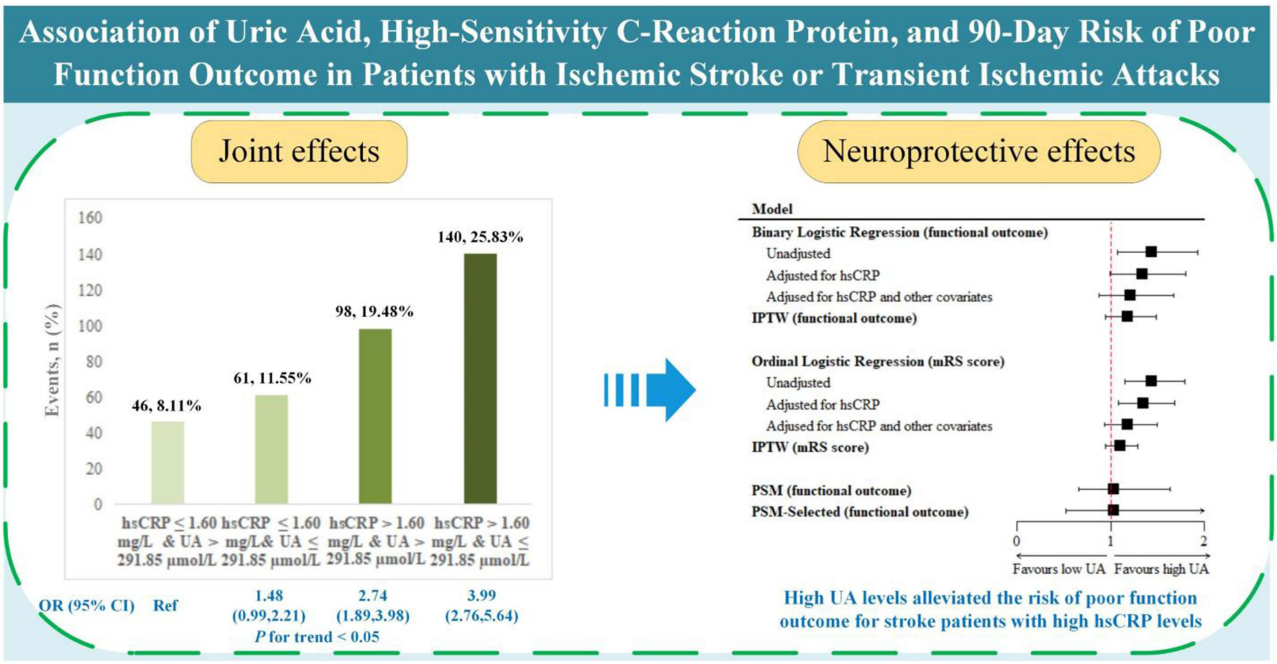
Graphical Abstract



profound, often resulting in significant neurological deficits that adversely impact patients' quality of life. Survivors frequently experience challenges in mobility, speech, and cognitive function, which can hinder their ability to perform daily activities and maintain independence.[3] And the primary pathological basis of most ischemic strokes is atherosclerosis, supported by four key lines of evidence.[4] Moreover, accumulating evidence supports the inflammation is of key importance in the pathophysiology of atherosclerosis.[4–6] Many previous studies have reported that inflammatory biomarkers such as IL-1, IL-6, TNF-α and hsCRP are associated with an increased risk of poor prognosis of patients with ischemic stroke or TIA.[5,7,8] Among these, hsCRP is a widely studied non-specific inflammatory biomarker for ischemic stroke, and high levels are independently linked to an increased risk of recurrent stroke, mortality, and poor functional outcomes in patients.[9] Our previous study also found that for each standard deviation increase in the concentration of hsCRP, there was an increased risk of functional disability within 90 days.[10]

In addition to inflammation, oxidative stress (OS) is also the pathogenesis of stroke.[11] The current two approved treatments (pharmacological thrombolysis and mechanical thrombectomy) for ischemic stroke to reperfusion of the ischemic region always lead to a highly harmful reactive oxygen species (ROS) and reactive nitrogen species (RNS) production,[12] but over-accumulation of ROS and RNS can generate OS and further to directly damage brain tissue.[11,13] Furthermore, the OS may be responsible for initiating an inflammatory process.[14] In addition to recanalization, another reasonable treatment alternative is to use compounds designed to protect brain cells during ischemia.[15] Uric acid (UA), a potent endogenous antioxidant for scavenging the OS agents (ROS and RNS), has been shown to exert strong neuroprotective effects in preclinical ischemic stroke models.[15,16] However, the relationship between UA and the prognosis of ischemic stroke in real-world studies is controversial because it has another deleterious effect, a pro-inflammatory effect.[17,18] It is the main reason that it consistently fails to enter the clinical arena. Previous randomized clinical trials (RCT) (URICO-ICTUS) of exogenous administration of uric acid to improve functional prognosis in patients with acute ischemic stroke also did not yield statistically different results.[19] Considering that about half of all ischemic stroke patients currently do not benefit from reperfusion therapy, it makes sense to reassess the neuroprotective role of uric acid based on real clinical data.[20] Moreover, given the close link between oxidative stress and inflammation,

further examining the relationship between UA and hsCRP, as well as the neuroprotective effects of UA in ischemic stroke patients with high hsCRP levels, is clinically significant. This could enhance our understanding of UA's potential clinical applications.

In general, the main aims were (1) to investigate whether hsCRP and UA have individual and joint effects on the 90-day functional outcome of ischemic stroke or TIA patients; and (2) to further explore the neuroprotective effects of UA in high levels of hsCRP subgroup patients, and in this analysis, we also utilized some propensity score models to reduce the influence of confounding factor to mimic some of the particular characteristics of the RCT.

Methods

Study Design and Participants

Our study initially included 5163 patients who presented with ischemic stroke or TIA between April 2015 and August 2018 at Beijing Tiantan Hospital (China National Clinical Research Center for Neurological Diseases). In short, participants were consecutively enrolled if meeting the following criteria: (1) admission with the diagnosis of ischemic stroke or TIA; (2) pre-stroke mRS score ≤ 2. This article conforms to the Strengthening the Reporting of Observational Studies in Epidemiology checklist.[21]

Baseline Data Collection and Measurements of Biomarkers

The trained nurses through face-to-face interviews or medical records to obtain patients' demographics (including age, sex, weight, height, prestroke mRS score, status of smoking and alcohol consumption), medical history (including diabetes, hypertension and hyperlipidemia), and hospitalization characteristics (including total costs and total time). The status of smoking and alcohol consumption were both categorized as "current", "previous" and "never": the "current" represented the individual was an active smoker/drinker at the time of stroke; the "previous" was defined as that the individual had quit smoking/drinking 1 year prior to the stroke; the "never" signified that the individual had never smoked/drank prior to the stroke. In addition, body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters.

Based on our previous studies on the association of inflammatory biomarkers and stroke outcomes,[22,23] we a priori included four of the biomarkers: hsCRP, UA, low-density lipoprotein cholesterol (LDL-C), and homocysteine (Cys). Laboratory tests of all biomarkers were performed within 24 h after admission at fasting condition in the central laboratory of Beijing Tiantan Hospital.

Follow-Up and Outcomes

The follow-up procedure of the study population was based on our concurrent study (Third China National Stroke Registry, CNSR-III).[24] Briefly, participants were followed up by trained research personnel through face-to-face or telephone interview in about 90 days. The primary follow-up information included patient's mRS score ranging from 0 (no symptoms) to 6 (death) and the administration of an antiplatelet drug during the follow-up period.

The primary outcome was a poor functional outcome defined as the mRS score of greater than 2 of all patients; for the high levels of hsCRP subgroup patients, the shift between the two different UA levels groups from one point category to another on the mRS score scale was regarded as the secondary outcome. The definition of the above-mentioned outcomes was consistent with studies of URICO-ICTUS and CNSR-III.[19,22]

Statistical Analysis

Distribution of all continuous variables was skewed based on Kolmogorov–Smirnov tests. We presented continuous variables as medians (interquartile ranges) and categorical variables as numbers (percentages). The nonparametric Wilcoxon test or Kruskal–Wallis test was utilized to compare group differences for continuous variables or ordinal variables, and chisq tests for categorical variables. We used the median of biomarkers (UA, LDL-C, and Cys) and the mode of other categorical covariates (diabetes and hypertension) to impute their missing values, respectively.

In our study, the statistical analysis plan consisted of two parts corresponding to the overall patients and patients with high level of hsCRP.

For overall patients, we explored the associations between biomarkers and follow-up outcomes in term of individual and joint effects, respectively. Individual effects: firstly, each biomarker (hsCRP, UA, LDL-C, Cys) was considered a categorical variable according to its quartiles. We evaluated the associations between biomarkers and primary outcome using the multivariate logistic regression models (MLR), comparing the first quartiles with the fourth quartiles of each biomarker. Secondly, the pattern and magnitude of correlation between each biomarker on a continuous scale and the primary outcome using MLR with restricted cubic spline. Three knots were set at the 5th, 50th and 90th percentiles and the reference value (OR = 1) was set at the median of each biomarker levels. Thirdly, hsCRP and UA were used as binary categorical variables (based on their median) for measuring the interaction of hsCRP and UA in the MLR by additive-scale (including relative excess risks due to interaction, RERI; attributable proportion due to interaction, AP; the synergy index, SI) and multiplicative-scale interaction.[25] In this analysis, if the 95% CI calculated for UA and hsCRP in the product term of the MLR did not contain 1, it indicated a multiplicative-scale interaction between them, and if the 95% CI for RERI, AP did not contain 0 and SI did not contain 1, the additive-scale interaction between UA and hsCRP existed. Variables including demographics, medical history, hospitalization and follow-up characteristics (antiplatelet drug) were adjusted in all MLR models. Joint effects: the "individual effects" section showed that patients with higher hsCRP quartile were at higher risk of poor functional outcome, whereas the opposite was true for UA. Therefore, based on the result of the "individual effects" section, patients were classified into four groups according to the median of hsCRP and UA to analyze the effect of combined biomarkers on primary outcome. Specially, group 1: hsCRP ≤ 1.60 mg/L and UA > 291.85μmol/L, group 2: hsCRP ≤ 1.60 mg/L and UA ≤ 291.85μmol/L, group 3: hsCRP > 1.60 mg/L and UA > 291.85μmol/L, group 4: hsCRP > 1.60 mg/L and UA ≤ 291.85μmol/L. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CIs) for the associations of hsCRP and UA with primary outcome, setting the group 1 as the reference group. We additionally set the cutoff values for hsCRP to the upper quartiles and 3 mg/L, referring to our previous study and the Centers for Disease Control and the American Heart Association's recommended cut point for hsCRP, respectively.[26,27] In addition, the lower quartiles of UA was another cutoff values for its. Totally, four sensitivity analyses were conducted with different combinations of hsCRP and UA.

For patients with high levels of hsCRP (ie, group 3 and group 4 in the "joint effects" section), the binary logistic regression (BLR) was conducted to assess the association between different levels of UA and primary outcome, and the ordinal logistic regression (OLR) was used to estimate the common odds ratio for a shift in the direction of a poor outcome on the mRS score (secondary outcome). The unadjusted and adjusted OR (BLR) or common OR (OLR) and their 95% CIs were calculated. Also, subgroup analysis was conducted to explore whether the association between different levels of UA and primary outcome was modified by covariates in the BLR. In order to achieve a well-balanced comparison between patients with low and high UA levels, the propensity score (PS) was calculated by logistic regression model based on eight variables (BMI, sex, age, smoking, alcohol, diabetes, hypertension and hyperlipidemia) to each patient.[28] Then, the PS-based inverse probability of treatment weighting (IPTW) was utilized to ensure the robustness of OLR and BLR. Furthermore, we modeled the propensity score matching (PSM) with 1:1 nearest neighbor matching in an attempt to compare it with the URICO-ICTUS finding. In the URICO-ICTUS study, patients treated with exogenous UA had an average increase in serum UA concentrations of more than 118.97 μmol/L.[19] So we additionally selected the matched pairs with a difference in the UA levels of 118.97μmol/L or more to perform PSM analysis (named PSM-Selected). The absolute standardized mean differences (SMDs) less than 0.25 were set as an indication to test the balance of covariates in matched pairs in PSM.

Overall, a 2-side $P < 0.05$ was considered to indicate statistical significance. SAS (version 9.4, SAS Institute, Inc, Cary, Nc) and R (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria) software were used for all statistical analysis.

Results

Patients Characteristics

Of the 5163 stroke patients followed up for approximately 90 days, 3147 patients with the prestroke mRS score ≤ 2 were diagnosed as ischemic or TIA, which, when combined with other available electronic health record information, resulted in a total of 2140 patients enrolled in the study. Characteristics of patients after stratification by primary outcome were shown in Table 1. The median age was 58 (IQR, 50–65), and 1673 (78.2%) patients were men in overall patients.

Table 1 Characteristics of Patients According to Primary Outcome

Variables	Total	Favorable Functional Outcome		*P* value
		Yes	No	
Demographics				
Age				<0.001
N	2140	1795	345	
Median (IOR)	58(50,65)	57(49,65)	61(55,68)	
Sex, n (%)				0.167
Male	1673(78.2%)	1413(78.7%)	260(75.4%)	
Female	467(21.8%)	382(21.3%)	85(24.6%)	
BMI				0.620
N	2140	1795	345	
Median (IOR)	25.4(23.3,27.7)	25.5(23.2,27.7)	25.4(23.4,28.0)	
Pre-stroke mRs score				0.726
N	2140	1795	345	
Median (Range)	0(0–2)	0(0–2)	0(0–2)	
Smoking, n (%)				0.295
Previous	369(17.2%)	305(17.0%)	64(18.6%)	
Never	777(36.3%)	643(35.8%)	134(38.8%)	
Current	994(46.4%)	847(47.2%)	147(42.6%)	
Alcohol consumption, n (%)				0.355
Previous	227(10.6)	185(10.3%)	42(12.2%)	
Never	871(40.7%)	725(40.4%)	146(42.3%)	
Current	1042(48.7%)	885(49.3%)	157(45.5%)	
Medical history				
Hypertension, n (%)				<0.001
Yes	1221(62.6%)	995(60.9%)	226(71.3%)	
No	730(37.4%)	639(39.1%)	91(28.7%)	
Missing	189	161	28	
Diabetes, n (%)				<0.001
Yes	540(27.7%)	420(25.7%)	120(37.9%)	
No	1411(72.3%)	1214(74.3%)	197(62.1%)	
Missing	189	161	28	
Hyperlipidemia, n (%)				0.746
Yes	351(18.0%)	296(18.1%)	55(17.4%)	
No	1600(82.0%)	1338(81.9%)	262(82.6%)	
Missing	189	161	28	
Hospitalization characteristics				
Total costs				<0.001
N	2140	1795	345	
Median (IOR)	18806.9(15,277.9,23,392.9)	18,312.1(14,996.1,22,234.6)	22,907.5(18,143.8,27,985.0)	
Total time				<0.001
N	2140	1795	345	
Median (IOR)	13(10,14)	13(10,14)	14(12,16)	

(*Continued*)

Table 1 (Continued).

Variables	Total	Favorable Functional Outcome		*P* value
		Yes	No	
Follow-up characteristics				
Antiplatelet drug				<0.001
Yes	1873(87.5%)	1591(88.6%)	282(81.7%)	
No	267(12.5%)	204(11.4%)	63(18.3%)	
Blood biomarkers				
hsCRP				<0.001
N	2140	1795	345	
Median (IOR)	1.6(0.7,4.4)	1.4(0.6,3.7)	3.6(1.2,10.2)	
UA				<0.001
N	2107	1765	342	
Median (IOR)	290.8(241.2,356.0)	293.4(244.0,358.8)	275.1(230.0,336.5)	
LDL-C				0.297
N	2111	1769	342	
Median (IOR)	2.1(1.7,2.7)	2.1(1.7,2.7)	2.0(1.7,2.6)	
Cys				0.426
N	2041	1713	328	
Median (IOR)	13.7(11.0,17.9)	13.6(11.0,17.9)	14.0(10.9,18.0)	

Abbreviations: BMI, body mass index; IQR, interquartile range; pre-stroke mRS, modified Rankin Scale prior to stroke; hsCRP, high-sensitivity C-reactive protein; UA, uric acid; LDL-C, low-density lipoprotein cholesterol; Cys, homocysteine.

Medical history of diabetes and hypertension was statistically significant between the two functional outcome groups, and the higher hsCRP and lower UA were observed in the poor functional outcome group (345, 16.1%).

Association of Individual and Joint Effects of hsCRP and UA with Poor Functional Outcome

In the analysis of "individual effects", the third quartiles (adjusted OR 1.563; 95% CI 1.059–2.305) and fourth quartiles of hsCRP (adjusted OR 3.090; 95% CI 2.150–4.442) were associated with increased risk of poor functional outcome, while the highest quartiles of UA (adjusted OR 0.671; 95% CI 0.459–0.883) was significantly correlated with better functional outcome (Figure 1). In addition, the risk of poor functional outcome significantly increased in older patients who had a higher BMI, had diabetes, and were not taking antiplatelet drug during follow-up (Figure 1). Similar results were observed when the hsCRP and UA as continuous variable in the MLR with restricted cubic spline (Supplementary Figure S1): the dose–response relationship of hsCRP and poor functional outcome was positively correlated, whereas UA was negative. The additive-scale and multiplicative-scale interaction of hsCRP and UA was not observed for the primary outcome: RERI = 0.724 (95% CI, −0.326–0.776, $P>0.05$), AP = 0.184 (95% CI, −0.070–0.437, $P>0.05$), SI = 1.323 (95% CI, 0.847–2.076, $P>0.05$), and multiplicative interaction = 0.973 (95% CI, 0.590–1.600, $P>0.05$).

In the analysis of "joint effects", Figure 2 illustrated the distribution of the scores of the mRS at 90 days of the four groups. The percentage of patients with poor functional outcome (mRS ≥ 2) increased steadily over group 1 to group 4 and there were differences in the statistical distribution between the four groups (Kruskal–Wallis $P < 0.001$). In the logistic regression analysis, the risk of poor functional outcome significantly increased in group 3 and group 4 compared those with group 1 (hsCRP ≤ 1.60 mg/L and UA > 291.85μmol/L), the adjusted OR (95% CI) was 2.740 (1.886–3.982) and 3.994 (2.758–5.640), respectively (Figure 3). Robust results were observed in sensitivity analysis (Figure 3).

Association of Different Levels of UA and Outcome in the Patients with High Levels of hsCRP

For those patients with high levels of hsCRP, patients with lower UA levels had an increased risk of poor functional outcome in unadjusted BLR (unadjusted OR, 1.439; 95% CI 1.074–1.929; P=0.015) (Figure 4); Similar results were

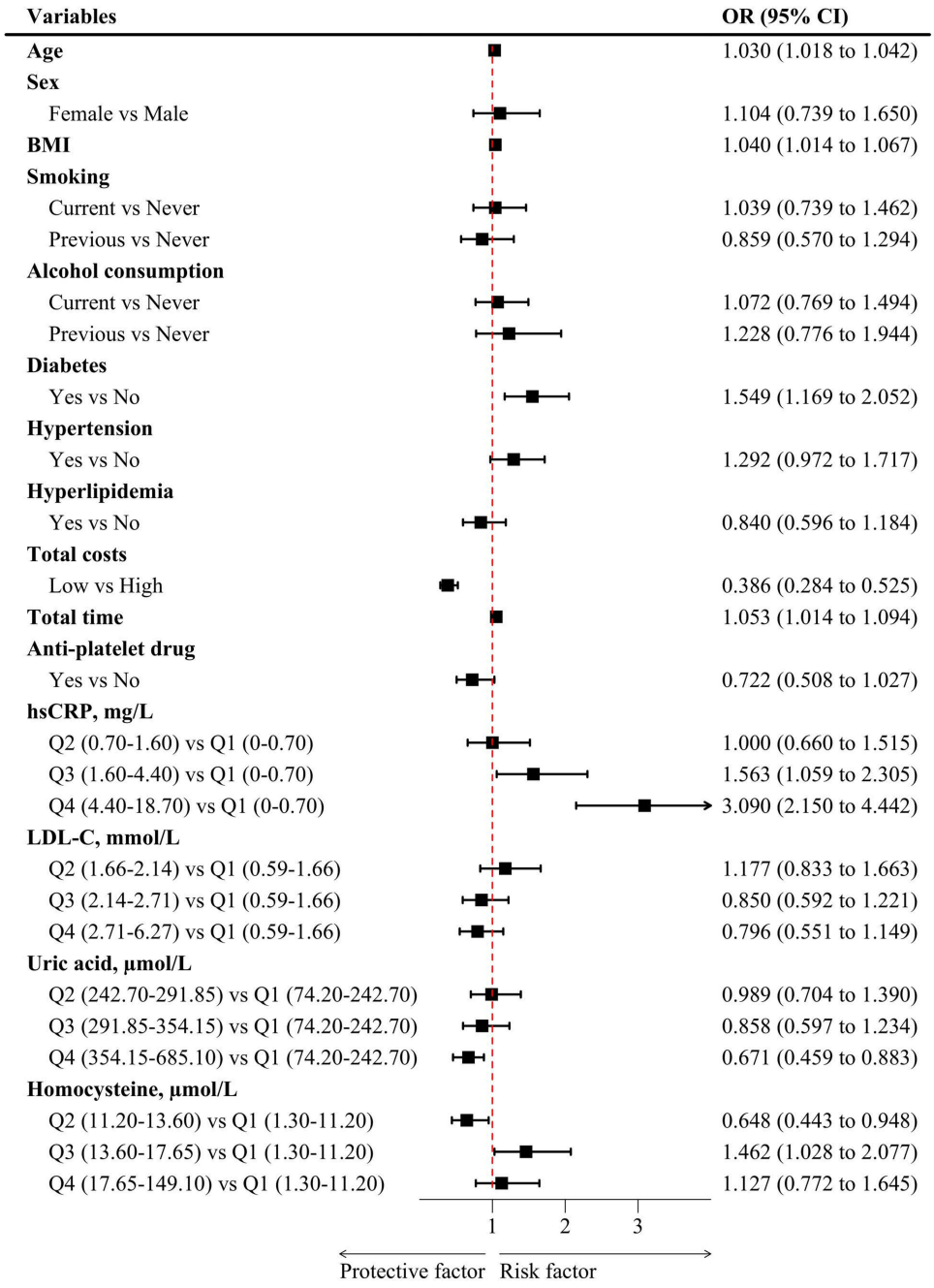


Figure 1 Multivariate logistic regression of biomarkers with functional outcome at 90 days. Adjusted odds ratio (95% CI) was calculated after adjusting for age, BMI, smoking, alcohol consumption, diabetes, hypertension, hyperlipidemia, total costs, total time and anti-platelet drug.
Abbreviations: BMI, body mass index, hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

observed in the OLR: patients with higher UA levels were in a better score category on the mRS (unadjusted common OR = 1.435, 95% CI 1.150–1.791, *P*=0.001; adjusted for hsCRP common OR = 1.349, 95% CI 1.080–1.685; *P*=0.008) (Figure 4). The results of the IPTW also showed potentially beneficial effects of high levels of UA on the distribution of mRS score (adjusted common OR = 1.208, 95% CI 1.035–1.411, *P*=0.016) (Figure 4), although there was a non-significant beneficial effect on functional outcomes (adjusted OR = 1.185, 95% CI 0.948–1.482, *P* = 0.135) (Figure 4). In the subgroup analysis, being female (adjusted OR=2.273, 95% CI 0.933–5.199, *P* = 0.051) and taking antiplatelet drug (adjusted OR = 2.301, 95% CI 0.997–5.311, *P* = 0.051) was marginally significant with a poor functional outcome (Supplementary Figure S2).

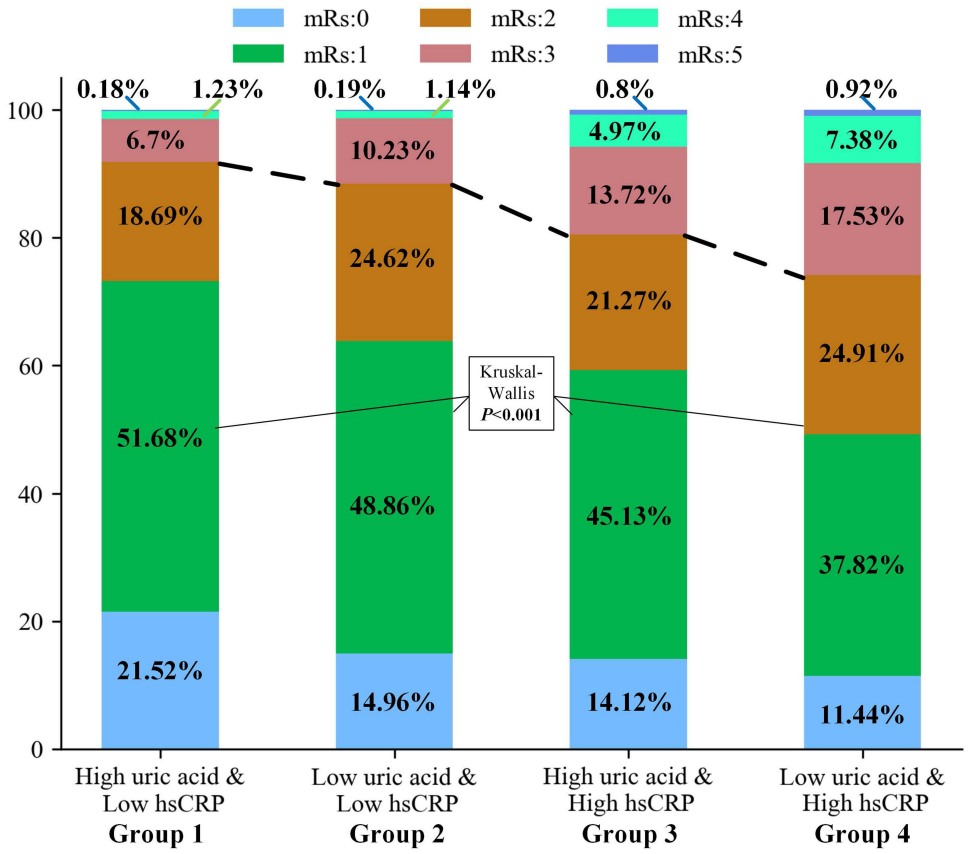


Figure 2 Distribution of scores on the mRS at 90 days for group 1 to group 4. Overall patients were divided into 4 groups based on the median of hsCRP and UA: group 1: hsCRP ≤ 1.60 mg/L and UA > 291.85μmol/L, group 2: hsCRP ≤ 1.60 mg/L and UA ≤ 291.85μmol/L, group 3: hsCRP > 1.60 mg/L and UA > 291.85μmol/L, group 4: hsCRP > 1.60 mg/L and UA ≤ 291.85μmol/L.
Abbreviations: mRS, modified Rankin Scale; hsCRP, high-sensitivity C-Reactive protein.

As for the PSM analysis, Supplementary Table S1 showed that all variables except BMI were similar between the two groups at different levels of UA after PSM. Absolute SMDs for all variables were less than 0.25 and also represented that the baseline profiles were well balanced after PSM (Supplementary Figure S3). Though the results of PSM and PSM-Selected not suggested that higher levels of UA were associated with decreased risk of poor functional outcome (Figure 4), the distribution of the mRS score remained statistically different between low and high levels of UA groups for the PSM patients and PSM-Selected patients, and the percentage of good functional outcome was higher in the high levels of UA group (Wilcoxon $P < 0.05$) (Figure 5).

Discussion

Previous studies have found both hsCRP and UA to be risk factors for poor prognosis in stroke patients, but unlike the well-verified hsCRP, conclusions from studies on UA and prognosis in stroke patients have been inconsistent.[7,9,18] In addition, there is a paucity of data aimed at exploring the relationship between hsCRP and UA (eg, interactions and joint effect) and poor functional outcome in stroke, and the neuroprotective effects of UA in patients with high levels of hsCRP also need to be explored.[20] In the current study, we observed that patients with joint high levels of hsCRP and low levels of UA had a higher risk of poor functional outcome at 90 days. Further analysis found that the high levels of UA were associated with a decreased risk of poor functional outcome in patients with high levels of hsCRP.

Inflammation plays an important part in the pathophysiology of ischemic stroke, and inflammatory biomarkers have been studied for a long time as possible prognostic markers for stroke patients.[29] Inflammatory biomarkers may contribute to the development of atherosclerosis by modulating cytokines, macrophages, and leukocyte adhesion molecules to induce endothelial dysfunction, plaque formation and rupture, platelet aggregation, and thrombosis.[30,31]

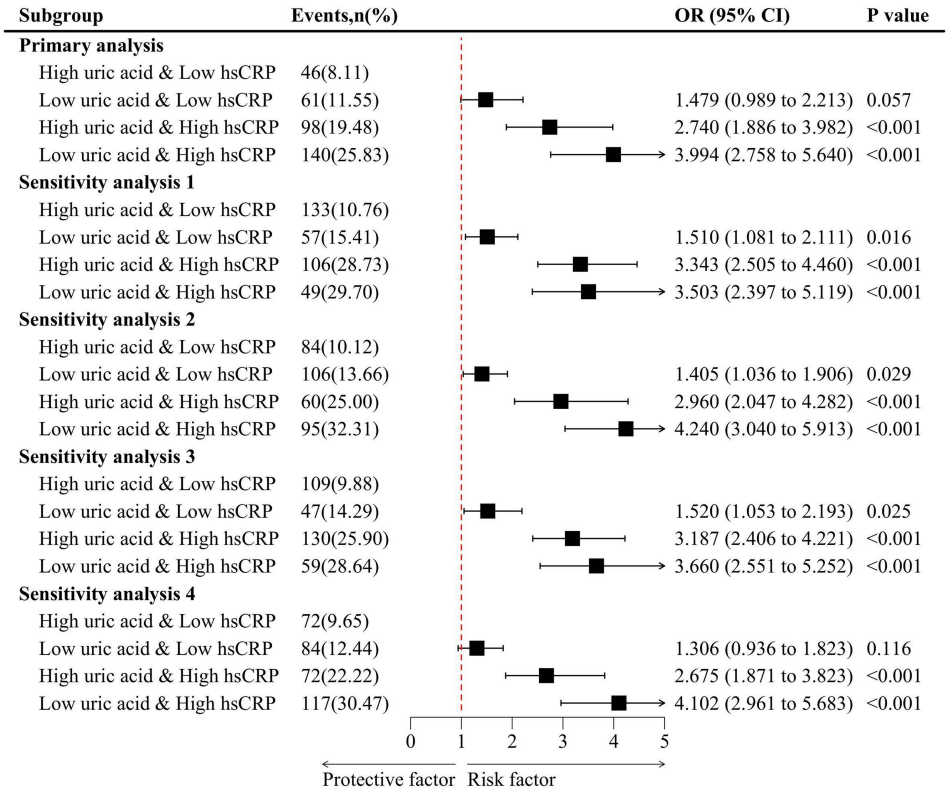


Figure 3 Analysis of joint effects of high-sensitivity C-reactive protein (hsCRP) and uric acid (UA) with primary outcome. Patients were divided into 4 groups and the first group as the reference group. The primary analysis and sensitivity analysis 1–4 from different combination cutoffs of hsCRP and UA. The primary analysis: 1.60 mg/L for hsCRP, 291.85 μmol/L for UA; sensitivity analysis 1: 4.40 mg/L for hsCRP, 242.7 μmol/L for UA; sensitivity analysis 2: 4.40 mg/L for hsCRP, 291.85 μmol/L for UA; sensitivity analysis 3: 3.0 mg/L for hsCRP, 242.7 μmol/L for UA; sensitivity analysis 4: 3.0 mg/L for hsCRP, 291.85 μmol/L for UA. All models were unadjusted for covariates.
Abbreviation: OR, odds ratio.

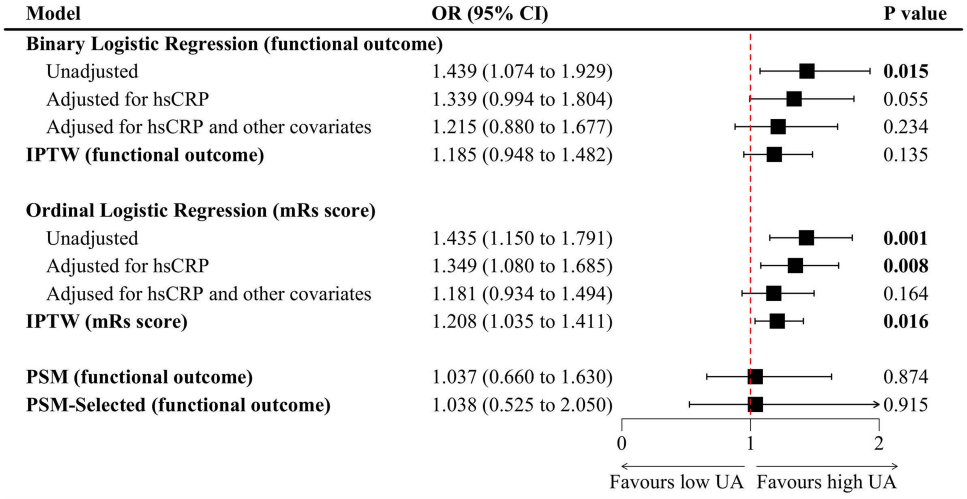


Figure 4 Association between different uric acid (UA) levels and primary and secondary outcomes in patients with high levels of high-sensitivity c-reactive protein (hsCRP). The binary logistic regression and ordinal logistic both included three models: model 1 was unadjusted; model 2 was adjusted for hsCRP; model 3 was adjusted for hsCRP and other covariates including age, BMI, smoking, alcohol consumption, diabetes, hypertension, hyperlipidemia, total costs, total time and antiplatelet drug. IPTW models additionally weighted the model 3 using weights calculated for each patient. PSM models were based on conditional logistic regression, adjusted for the same variables as in model 3.
Abbreviations: OR, odds ratio; IPTW, inverse probability of treatment weighting; PSM, propensity score matching; mRS, modified Rankin Scale.

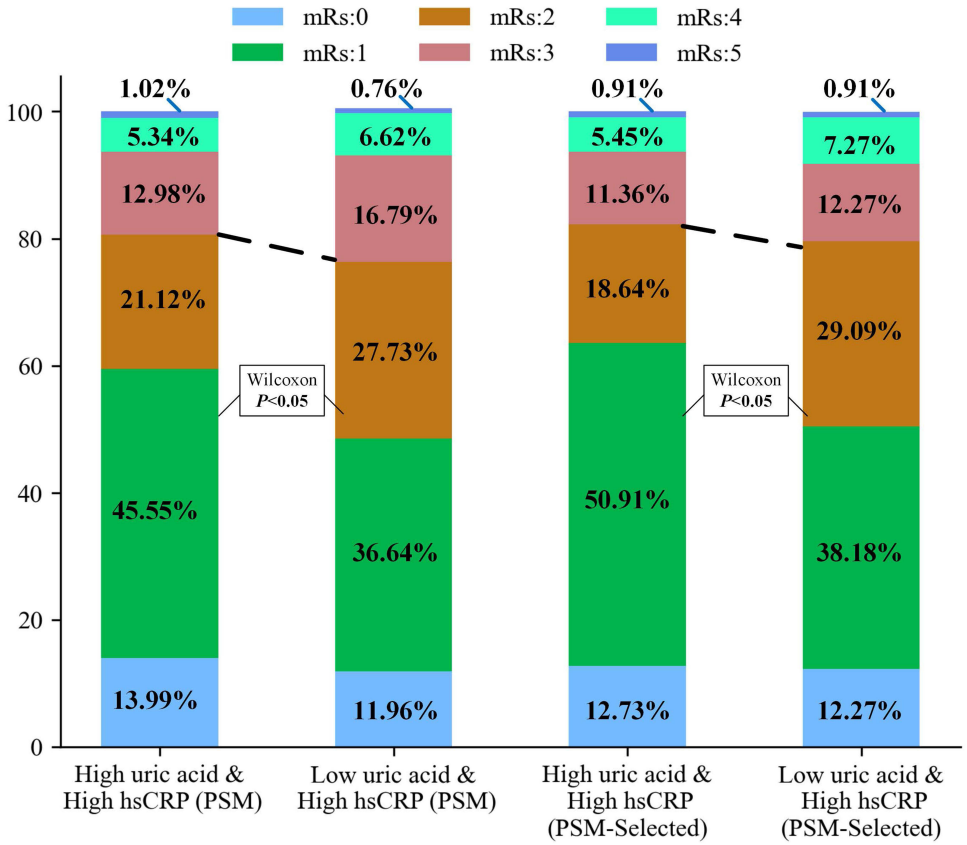


Figure 5 Distribution of scores on the mRS at 90 days for PSM and PSM-Selected patients.
Abbreviations: mRS, modified Rankin Scale, hsCRP, high-sensitivity C-Reactive protein.

HsCRP is a well-recognized biomarker of inflammation. But hsCRP, in addition to its atherogenesis-promoting effects described above, can also cause cell death, brain injury, and blood–brain barrier disruption to directly result in poor prognosis in stroke patients.[32,33] There is ample evidence to support that high levels of hsCRP are associated with poor prognosis in stroke patients.[9] A large meta-analysis study involving 11184 patients with acute ischemic stroke proved that the incidence of poor functional outcome in patients with high hsCRP levels was 1.77 of that in patients with low hsCRP levels (OR = 1.77, 95% CI, 1.59–1.97).[9] RCT studies have shown that lowering hsCRP levels can improve prognosis: Canakinumab, an anti-inflammatory drug, was proven to significantly reduce the risk of recurrent atherosclerotic disease (including stroke) by reducing the hsCRP level.[34] In the "individual effects" of our study, after adjustment for covariates, these patients with higher hsCRP levels (1.6–4.4 mg/L and > 4.4 mg/L) had poor functional outcome at 90 days, and the dose–response relationship was relatively apparent in the RCS. Our previous study also found that patients with hsCRP >3mg/L had a higher risk of poor functional outcome compared with those with hsCRP <1 mL/L at 90 days (OR = 1.68, 95% CI, 1.22–2.32).[7] LDL-C and Cys as prognostic markers for ischemic stroke were included into our analysis. They were both considered as biomarkers of inflammation, and previous studies have revealed their effects alone, as well as combined effects with hsCRP on poor prognosis in ischemic stroke.[22,23] However, there was no significant association between LDL-C and Cys with poor functional outcome at 90 days in our study, which still needs to be determined in future studies.

In addition to inflammation, the OS is also strongly associated with poor prognosis in ischemic stroke. The OS is an imbalance between oxidative and antioxidative systems and can be caused by excessive production of ROS and RNS during cerebral ischemia.[35] After ischemia, the brain will have an ischemic penumbra in a hypoperfused state between the irreversible central zone and normal brain tissue. Conversion of the ischemic penumbra into a normally perfused area, followed by the construction of new neurofunctional connections through residual neurons in the penumbra, is the main

cause of functional recovery after stroke.[36] However, the occurrence of OS will cause an ischemic cascade response, which will promote the poor development of the ischemic penumbra, leading to poor recovery of stroke neurological function.[37] In particular, the post-ischemic brain is in a state of high oxygen depletion, rich content of iron, unsaturated lipids, and low antioxidant capacity, making it more susceptible to damage by OS.[38] In addition, the re-entering of oxygen and glucose into the ischemic brain at reperfusion facilitates an excess production of ROS and RNS, leading to reperfusion injury. Therefore, the antioxidant therapy to prevent the effects of RNS and ROS is a potential therapeutic strategy. UA is an end-product of purine catabolism and accounts for as much as two-thirds of the total plasma antioxidant capacity. It can act as a potent endogenous antioxidant to scavenge the OS agents: the UA has been shown to suppress ROS and RNS to improve functional outcome after transient or permanent brain ischemia in rodents.[16,39] An only RCT of UA in human was a phase 2b/3 URICO-ICTUS trial: though the addition of UA to thrombolytic therapy did not increase the proportion of patients who achieved excellent outcome (mRS score at 90-day follow-up) in this RCT, UA did reduce the incidence of early clinical deterioration, and more patients treated with UA achieved full independence at follow-up.[19] Our analysis of individual effect in UA found that UA at the fourth quartile vs the first quartiles (adjusted OR = 0.671, 95% CI, 0.459–0.883) was associated with a decreased risk of poor functional outcome at 90 days. Another multicenter stroke registry study also observed that decreases in UA levels were independently associated with unfavorable outcomes after ischemic stroke.[40] It is undeniable that real-world studies on whether UA has neuroprotective effects are inconsistent. That is because the UA can exert pro-inflammatory effect on atherosclerosis other than the antioxidant effects.[41] It had been reported that elevated uric acid increased free radical production, promoted LDL-C oxidation (oxLDL), and raised levels of inflammatory cytokines, which exacerbated the level of inflammation and the progression of atherosclerosis.[42] Spearman correlation analyses showed that biomarkers levels were mildly correlated (defined as a Spearman correlation coefficient r<0.3, Supplementary Table S2). Moreover, in contrast to our result of the RCS, which showed a negatively linear dose–response association between UA and primary outcome (but not significant), the association between UA levels and clinical outcomes in ischemic stroke in relevant studies showed discrepant findings, such as U-shaped or J-shaped relationships.[43,44] These inconsistent findings may be due to whether the primary role of uric acid in these studies was pro-inflammatory or antioxidant, as well as the differently defined subgroup of patients (ie, sex or diabetes).[15] However, since the 95% CI of the RCS results for high UA levels contained 1, we should be cautious about the linear correlation.

In fact, the OS and inflammation are closely linked, and they affect each other. In the case of OS, the onset of OS leads to endothelial dysfunction, which is thought to be the first step in the formation of atherosclerotic plaques and localized inflammation.[45,46] A recent study found that oxLDL treatment induced the OS and inflammatory response in human aortic endothelial cells, linking the endothelial dysfunction, oxidative stress, inflammation and atherosclerosis from a molecular perspective.[47] However, to the best of our knowledge, evidence on the interaction and combined effects between hsCRP and UA on poor prognosis is not available. In the current study, it was worth pointing out that although we did not find a statistically significant interaction between UA and hsCRP, this did not mean that there was not a biologically significant interaction between them. Instead of focusing a single biomarker, the "joint effects" analysis investigated the joint effect of UA and hsCRP on poor functional outcome. We used the median of hsCRP and UA to differentiate between high and low risk, and found that the strength of the association of hsCRP and UA with poor functional outcome was higher in the groups that either or both of them were at high risk, when both low risk treated as the reference group. Sensitivity analysis of different cutoff combinations of hsCRP and UA also yielded consistent results. These findings suggested that the combination of hsCRP and UA were potent biomarkers of poor functional outcome after ischemic stroke or TIA, which was beneficial for stroke risk stratification and identification of high-risk subgroups.

Previous study pointed that the encouraging results obtained with UA treatment in URICO-ICTUS warrant validation in future study, especially in special subgroup patients.[15] In the second analysis of UCICO-ICTUS, patients treated with UA who were female, pretreatment hyperglycemia or early recanalization could reach better prognosis than placebo.[48,49] Therefore, in our prospective subgroup cohort (patients with high hsCRP levels), we explored the association between different UA levels (high vs low) and poor functional outcome at 90 days to examine the UA's neuroprotective effects. For both the primary and secondary outcomes, we observed a significant (P<0.05) /weak (P=0.055) correlation between high UA levels and excellent

outcome, unadjusted or adjusted for hsCRP. However, those correlations disappeared after adjusting for all covariates. We speculated that unbalanced covariates between the high and low UA levels groups (Supplementary Table S1) thwarted the effects of UA. Some, therefore, PS-based models (IPTW and PSM) were conducted to cope with the confounding bias. The PS is a powerful tool for balancing confounders to achieve efficacies comparable of non-randomized control trials and has a wide application prospect.[28,50] Though no significant association between UA and outcomes was reached in the results of the IPTW, we can observe that the 95% CI of OR in the IPTW was narrower compared with counterparts of the OLR and BLR adjusted for all covariates, and thus the reliability of the parameter estimates for IPTW was higher, which strengthened our confidence that increased uric acid levels may be associated with a reduced poor functional prognosis after stroke. In addition, since reduced sample size after PSM, insufficient sample size might cause the 95% CI for OR in PSM models was wider with lower reliability. Whatever, although our different analytical approaches did not yield identical results, we believed that we have revealed the potential for therapeutic application of UA in stroke patients with high hsCRP levels. Furthermore, subgroup analyses of sex and usage of antiplatelet drug also underlined differences in the beneficiary stroke patients from high UA levels, with the results of sex consistent with those of URICO-ICTUS, and our previous study also found the use of antiplatelet drug during follow-up was beneficial for patients with ischemic stroke or TIA.[19,22] Overall, our data at least have potential importance for the design of future UA trials aimed at exploring the neuroprotective effects of UA in stroke patients.

The strength of this study was that we more comprehensively explored the relationship between hsCRP and UA with prognosis after ischemic stroke or TIA. However, this study also had some limitations. First, all biomarkers were obtained by fasting venous blood collection after hospital admission, so their acute levels could not be recorded. Second, we only included one point measurements of markers at the time of hospitalization so were unable to assess the effect of changes in the levels of these markers over time and the hsCRP and UA levels during follow-up were not recorded in this study. Third, although we performed PS-based models to adjust for unbalanced covariates in this study, there might be unmeasured covariates and selection bias. Fourth, all participants were Chinese and this study was a single-center study, so our findings may not be generalizable to other races and ethnicities.

Conclusions

In patients with ischemic stroke or TIA, the joint effects of high hsCRP levels and low UA levels on poor functional outcomes were notably significant, which could enhance risk classification in clinical practice. Additionally, high UA levels were associated with improved prognostic outcomes for patients with elevated hsCRP. Based on these findings, we recommend considering the inclusion of UA levels in the routine monitoring of stroke patients. However, confirmatory trials are necessary to further evaluate the benefits of UA therapy.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki. This study was approved by the Institutional Review Boards (IRB) of Beijing Tiantan Hospital (IRB approval number: KY2015-001-01). Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable data included in the article.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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