High serum complement component C4 as a unique predictor of unfavorable outcomes in diabetic stroke

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Received: 27 February 2021 / Accepted: 24 August 2021 / Published online: 4 September 2021
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Abstract
Previous studies demonstrated that diabetic stroke patients had a poor prognosis and excess complement system activation in the peripheral blood. In this study, the association of serum complement levels with the prognosis of diabetic stroke was examined. Patients with acute ischemic stroke were recruited and were divided into two groups according to their history of diabetes. Baseline data on the admission, including C3 and C4 were collected. Neurologic function at discharge was the primary outcome and was quantified by the National Institutes of Health Stroke Scale (NIHSS). A total of 426 patients with acute ischemic stroke (116 diabetic strokes and 310 non-diabetic strokes) were recruited in this study. There were significant differences between the two groups in hypertension, coronary disease, triglyceride, high-density lipoprotein cholesterol, fasting blood sugar, C4, and mortality rates. Furthermore, the values of complement protein levels were divided into tertiles. In the diabetic stroke group, serum C4 level at the acute phase in the upper third was independently associated with NIHSS score at discharge and concurrent infection. These associations were not significant in non-diabetic stroke. High serum C4 level at admission, as a unique significant predictor, was associated with unfavorable clinical outcomes in the diabetic stroke, independently of traditional risk factors.

Keywords Complement system · C4 · Diabetes mellitus · Ischemic stroke · Outcome

Introduction
Stroke and diabetes mellitus (DM) are two complicated diseases that often occur together (Malla et al. 2019). Stroke worsens glucose metabolism abnormalities, and the outcomes after stroke are more severe for diabetic patients than those without diabetes (Forti et al. 2020; Lau et al. 2019). The overlapping risk factors and genetic data from multiple human cohorts for diabetes mellitus and cerebrovascular disease support the concept that the two diseases share common antecedents and critical pathogenic mechanisms (Bao et al. 2018; O'Donnell et al. 2016; Shu et al. 2017). Though inflammation is firmly established as central to both stroke and diabetes pathophysiology, the specific inflammatory processes involved may differ between them (Bao et al. 2018; Roth et al. 2018; Ruparelia et al. 2017; Saltiel and Olefsky 2017).

The complement system seems to play an important role in stroke (Alawieh et al. 2015a; Ma et al. 2019; Szeplaki et al. 2009). The three complement activation pathways are the classical pathway, the mannann-binding lectin pathway,
and the alternative pathway (Ghebrehiwet 2016). Elevated plasma levels of C3 and C5b-9 were positively correlated with the unfavorable clinic outcome (Mocco et al. 2006; Szeplaki et al. 2009). Meanwhile, complement component C4 is an essential part of the cascade that leads to C3 activation in the classical and mannann-binding lectin pathways; early steps in both pathways involve cleavage of C4 to C4b (Copenhaver et al. 2019). Compared with vascular disease, the relationship between the complement system and diabetes mellitus may be closer (Mellbin et al. 2012). Plasma levels of C3 are a more effective and more precise predictor of diabetes than multiple other acute-phase proteins (Bao et al. 2018; Borne et al. 2017). However, the potential prognostic value of these inflammatory markers may be influenced by ischemic stroke etiology. Existing data are still limited, and further investigation is required to explore the value of complement components for stroke patients, especially those with diabetes.

In the present study, we compared plasma levels of C3 and C4 in two groups of patients, diabetic stroke and non-diabetic stroke. We tried to distinguish their shared and specific markers and explore the mechanisms underlying the role of complement in diabetic stroke.

Material and methods

Study population

We conducted a retrospective review of patients who presented with first-ever or recurrent acute ischemic stroke (AIS) in the First Affiliated Hospital of Soochow University from January 2018 to October 2020. Participants were randomly selected from all eligible patients, resulting in a sample size that was one-sixth of the total eligible patients. Routine blood and biochemical tests, ECG, and a baseline brain CT/MRI scan were performed for all patients at admission. Laboratory investigations for vascular risk factors, duplex sonography of the carotid and vertebral arteries, and a thorough cardiac investigation were taken. Diabetes was defined as (1) with documented DM; (2) using diabetes medication; (3) fasting whole blood glucose ≥ 7.0 mmol/L and/or random whole blood glucose ≥ 11.1 mmol/L. Hypertension was defined as (1) the previous use of antihypertensive medication; (2) a systolic blood pressure ≥ 140 mmHg, and/or a diastolic blood pressure ≥ 90 mmHg. Atrial fibrillation was defined as (1) with documented atrial fibrillation; (2) electrocardiogram showed atrial fibrillation. The coronary disease was defined as (1) with documented coronary disease; (2) electrocardiogram showed definite Q-wave myocardial infarction; (3) echocardiographic showed (old) myocardial infarction; (4) coronary angiography showed coronary stenosis. Hyperlipidemia was defined as (1) fasting serum low density lipoprotein cholesterol (LDL-C) > 3.64 mmol/L; (2) fasting serum high density lipoprotein cholesterol (HDL-C) < 0.91 mmol/L; (3) fasting serum triglyceride (TG) > 1.70 mmol/L; (4) fasting serum total cholesterol (TC) > 5.72 mmol/L.

The initial sample included 620 patients. Exclusion criteria were as follows: (1) patients were younger than 18 years old; (2) patients with cerebral infarction caused by subarachnoid hemorrhage, sinus venous thrombosis, or severe head trauma; (3) patients had a stroke history within 6 months or the modified Rankin scale (mRS) > 0 before the onset; (4) patients had a history of infection within 2 weeks before admission that was defined as fever (T ≥ 38°C) and at least one other typical symptom (cough, rhinitis, hoarseness, sneezing, or vomiting); (5) Patients had a history of hematological diseases, autoimmune diseases, or treatment with immunosuppressive agents; (6) patients with missing data (no blood test result within 24 h of admission). Only 426 patients with AIS (116 diabetic strokes and 310 non-diabetic strokes) were included and formed the basis of this report (Fig. 1). According to the current guidelines, all patients received the same medical advice and treatment, including a reasonable diet, effective blood pressure control, and anti-platelet medicine.

Clinical information collection

Baseline data were collected, including gender, age, cerebral vascular risk factors such as hypertension and diabetes, through electronic patient records and administrative databases. Mortality was defined as death during hospitalization or within 3 days after withdrawing treatment. Peripheral venous blood samples were collected on the morning of the second day after admission with overnight fasting. Complement C3 and C4 were measured using Beckman Specific Protein (America, model: IMMAGE800). Reagents were bought in Beckman Coulter (USA) co. LTD (immune scattering turbidimetry). All test reagents are within the validity period, and the quality of the research instruments has been appropriately controlled. The data analyst was blind to the specific clinical situation of the sample.

Evaluation of outcome

The National Institutes of Health Stroke Scale (NIHSS), a 15-item neurologic evaluation that grades the severity of the stroke, was used to assess the patient’s neurologic function at admission and discharge. The poor outcome was defined as NIHSS score at discharge > 10 or death (NIHSS score = 42) (Mihindu et al. 2019; Schonenberger et al. 2016). The secondary outcome was a concurrent infection, including urinary tract infection, pneumonia, biliary tract infection, and/or digestive tract infections.
Data analyses

Continuous variables were analyzed as mean and standard deviation or the median and interquartile range, while categorical variables were analyzed as frequency and percentage, properly. The differences among continuous variables were analyzed by the Student’s t-test or the Mann–Whitney U test, while Chi-square test assessed differences among categorical variables. Pearson’s correlation coefficients were calculated to assess the relationship between the variables. Furthermore, the values of complement protein levels were divided into tertiles. Logistic regression analysis was used to investigate the association between complement levels and case status after adjusting for other variables selected from the established risk factors. Therefore, no prior power analysis was conducted to calculate the sample size. Considering the correlation between smoking status and gender in China, we did not bring them into the adjustment factors simultaneously. The level of significance for these descriptive comparisons was established at 0.05 for two-sided hypothesis testing. Statistical analysis was performed in SPSS 25.0.

Results

Participants and their demographics and characteristics

A total of 426 patients with AIS were included in our study; 116 (27.2%) of them were diabetic stroke, which
was consistent with previous meta-analysis results (Lau et al. 2019). Patient baseline characteristics were shown in Table 1. The average age of all patients was 68.0 (57.0, 75.0) years old; 299 (70.2%) were male, and the ratio of male to female was about 7:3. 174 (40.8%) patients had a smoking history; 301 (70.7%) had a hypertension history; hospitalization days was 14 (11, 17); NIHSS score at admission was 3.0 (1.0, 7.0) and NIHSS score at discharge was 1.0 (0.0, 6.0); concurrent infection rate, and mortality rate were 26.1%, 2.1%, respectively.

**Comparison of demographic and clinical characteristics**

Depending on whether the patient had diabetic or not, the participants were divided into two groups: the diabetic stroke group with 116 patients and the non-diabetic strokes group with 310 patients. Statistical analysis indicated significant differences in hypertension, coronary disease, TG, HDL, FBS, C4, and mortality rates between the two groups \( p < 0.05 \). However, there were no significant differences in age, gender, smoking, or drinking, TC, LDL, WBC, NLR, neutrophil count, NIHSS at admission and NIHSS score at discharge, concurrent infection rate, hospitalization days, C3, factor B, or other factors between the two groups \( p > 0.05 \), Table 2).

Furthermore, we calculated the correlation coefficients between C3, C4, WBC, NIHSS at admission and NIHSS at discharge. Observed correlation coefficients in diabetic stroke group: \( r = 0.580 \) between C3 and C4 \( p < 0.01 \); \( r = 0.199 \) between the C3 and WBC \( p < 0.05 \); \( r = 0.255 \) between C3 and NIHSS at admission \( p < 0.01 \); \( r = 0.213 \) between C4 and WBC \( p < 0.05 \). Observed correlation coefficients in non-diabetic stroke group: \( r = 0.508 \) between C3 and C4 \( p < 0.01 \); \( r = 0.213 \) between the C3 and WBC \( p < 0.01 \); \( r = 0.200 \) between C3 and NIHSS at admission \( p < 0.01 \); \( r = 0.142 \) between C4 and WBC \( p < 0.05 \); \( r = 0.140 \) between C4 and NIHSS at admission \( p < 0.01 \). C3 had weak but significant correlations with C4, WBC, and NIHSS at admission in both groups (Table 3).

**C4 was associated with poor outcomes in diabetic stroke**

Univariate and multivariable logistic regression analyses were used to determine factors that were significantly associated with poor outcomes after AIS. Values of cutoffs for C3 and C4 tertiles were listed in Table 4, and detailed results of binary logistic regression analysis of the case status are presented in Table 5 and Table 6. As the number of participants with mortality was minimal, no separate functional analysis could be made.

For the diabetic stroke group, acute phase plasma C4 concentration in the upper third was associated with NIHSS score at discharge in the multivariate analysis (adjusted OR, 11.262; 95%CI, 1.519–83.488; \( p < 0.05 \)). Models were established for a group of confounding factors: gender, TC, TG, HDL, FBS. Interestingly, this association was lost in the non-diabetic stroke group. Meanwhile, WBC, instead of C3, was associated with an unfavorable outcome in the upper third in both groups (Table 5).

In addition, our study results indicated that acute phase plasma C4 concentration in the upper third was associated with concurrent infection in the multivariate analysis (adjusted for gender, TC, TG, HDL, FBS; adjusted OR, 10.506; 95%CI, 2.265–48.733; \( p < 0.01 \)). This association was also lost in the non-diabetic stroke group. At the same time, C3 and WBC were associated with concurrent infection in the upper third in both groups (Table 6).
Table 2 Clinical and laboratory findings in patients with diabetic stroke and non-diabetic stroke

| Characteristics                  | Diabetic stroke (N=116) | Non-diabetic stroke (N=310) | p       |
|----------------------------------|-------------------------|-----------------------------|---------|
| Age in years, median (IQR)       | 69.00 (60.25, 73.00)    | 67.00 (57.00, 77.00)        | 0.992   |
| Male, n (%)                      | 87 (75.0)               | 212 (68.4)                  | 0.185   |
| Drinking, n (%)                  | 4 (3.45)                | 11 (3.55)                   | 1.000   |
| Smoking, n (%)                   | 50 (43.10)              | 124 (40.0)                  | 0.562   |
| Hypertension, n (%)              | 93 (80.20)              | 208 (67.10)                 | 0.008   |
| Atrial fibrillation, n (%)       | 9 (7.80)                | 39 (12.60)                  | 0.161   |
| Coronary disease, n (%)          | 9 (7.80)                | 8 (2.60)                    | 0.015   |
| Hyperlipidemia, n (%)            | 3 (2.60)                | 7 (2.30)                    | 0.842   |
| FBS in mmol/l, median (IQR)      | 7.41 (6.14, 9.34)       | 4.89 (4.47, 5.54)           | <0.001  |
| C3 in g/L, median (IQR)          | 0.89 (0.78, 0.98)       | 0.86 (0.76, 1.00)           | 0.353   |
| C4 in g/L, median (IQR)          | 0.23 (0.19, 0.27)       | 0.21 (0.18, 0.25)           | 0.045   |
| C3/C4, median (IQR)              | 3.98 (3.47, 4.42)       | 4.12 (3.50, 4.72)           | 0.103   |
| Factor B in mg/dL, median (IQR)  | 35.45 (29.93, 41.80)    | 36.25 (31.73, 42.53)        | 0.158   |
| WBC in ×10^9/L, median (IQR)     | 7.17 (5.87, 8.65)       | 6.82 (5.57, 8.66)           | 0.360   |
| L in ×10^9/L, median (IQR)       | 1.45 (1.10, 1.90)       | 1.58 (1.11, 1.99)           | 0.445   |
| N in ×10^9/L, median (IQR)       | 4.63 (3.77, 6.30)       | 4.56 (3.40, 5.97)           | 0.320   |
| NLR, median (IQR)                | 2.89 (2.15, 4.35)       | 2.78 (2.03, 4.50)           | 0.359   |
| TC in mmol/L, mean±SD            | 4.19±1.00               | 4.19±1.31                   | 0.953   |
| TG in mmol/L, median (IQR)       | 1.39 (1.08, 2.05)       | 1.23 (0.94, 1.63)           | 0.002   |
| HDL in mmol/L, median (IQR)      | 0.85 (0.73, 1.06)       | 0.95 (0.81, 1.14)           | <0.001  |
| LDL in mmol/L, median (IQR)      | 2.47 (1.77, 3.24)       | 2.52 (1.89, 3.17)           | 0.624   |
| Hospitalization days, median (IQR)| 14.00 (12.00, 17.00)   | 14.00 (11.00, 16.00)        | 0.087   |
| Mortality, n (%)                 | 5 (4.3)                 | 4 (1.3)                     | 0.054   |
| NIHSS at admission, median (IQR) | 3.00 (1.00, 7.00)       | 3.00 (1.00, 6.25)           | 0.437   |
| NIHSS at discharge, median (IQR) | 2.00 (0.00, 7.00)       | 1.00 (0.00, 5.00)           | 0.117   |
| Concurrent infection, n (%)      | 34 (29.30)              | 77 (24.80)                  | 0.349   |

Abbreviations: IQR, interquartile range; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; WBC, white blood cell; NIHSS, National Institute of Health Stroke Scale

Table 3 Pearson correlation coefficients between the variables

|             | C3       | C4       | NIHSS at admission | NIHSS at discharge | WBC       |
|-------------|----------|----------|--------------------|--------------------|-----------|
| Diabetic stroke |         |          |                    |                    |           |
| C3          | -        | 0.580**  | 0.255**            | 0.028              | 0.199*    |
| C4          | 0.580**  | -        | 0.098              | 0.047              | 0.213*    |
| NIHSS at admission | 0.255**  | 0.098    | -                  | 0.615**            | 0.245**   |
| NIHSS at discharge | 0.028    | 0.047    | 0.615**            | -                  | 0.513**   |
| WBC         | 0.199*   | 0.213*   | 0.245**            | 0.513**            | -         |
| Non-diabetic stroke |      |          |                    |                    |           |
| C3          | -        | 0.508**  | 0.200**            | 0.158**            | 0.213**   |
| C4          | 0.508**  | -        | 0.140*             | 0.125*             | 0.142*    |
| NIHSS at admission | 0.200**  | 0.140*   | -                  | 0.706**            | 0.398**   |
| NIHSS at discharge | 0.158**  | 0.125*   | 0.706**            | -                  | 0.415**   |
| WBC         | 0.213**  | 0.142*   | 0.398**            | 0.415**            | -         |

Values represent Spearman’s correlation coefficients (r) with significance levels were denoted as follows: * p < 0.05; ** p < 0.01

–indicates non-relevant correlation
Discussion

In our study, the plasma levels of C3 and C4 between diabetic stroke and non-diabetic strokes groups were analyzed to explore the similarities and differences between groups. The main results are as follows: (1) in the diabetic stroke group, high plasma C4 level at admission showed an association with patient prognosis independently of traditional risk factors; (2) in AIS patients, with or without diabetes, high plasma C3 levels were associated with concurrent infection. To our knowledge, this study was the first time to analyze the relationship between C4 and clinical outcomes in diabetic stroke patients.

Results from a number of studies point out that patients with diabetic stroke may have a poor prognosis, and the excessive activation of the complement system could be one of the reasons. As an important branch of the innate immune system, complement plays important roles in development, homeostasis, and regeneration in the central nervous system (CNS) (Carpanini et al. 2019). Given the roles of complement, enhancing complement activity may be of benefit in certain situations (Hammad et al. 2018). However, in the context of CNS pathology, complement dysregulation may lead to over-activation and contribute to neuroinflammation (Carpanini et al. 2019; Hammad et al. 2018; Mellbin et al. 2012; Szeplaki et al. 2009). During neuroinflammation, not only microglia and astrocytes, but also neurons, oligodendrocytes, and endothelial cells in the brain, can express complement components and receptors, many of which are upregulated by inflammatory signals (Carpanini et al. 2019; Lubbers et al. 2017). A recent study showed that complement expression was increased in cerebral ischemia/reperfusion injury of diabetic mice and complement deficiency abrogated the injury (Lin et al. 2018). Furthermore, the study found that activating complement promoted TLR2/NFkB activation after ischemia/reperfusion injury in diabetic mice, inhibited by the silencing of TLR2 (Lin et al. 2018). Gene expression profiling revealed that site-targeted complement inhibition could downregulate genes associated with apoptosis, TGFβ signaling, neutrophil activation, and decreased neutrophil infiltration (Alawieh et al. 2015b). In addition, as a result of increased blood brain barrier permeability, circulating neurotransmitters and large proteins such as complement are infiltrated into the injured brain during pathology (Carpanini et al. 2019; Ueno et al. 2016).

C3 and C4 are acute-phase proteins and important components of the complement pathways of the immune system (Ritchie et al. 2004). Even though many cell types express these proteins, including adipose tissue and vascular cells, hepatic production is the primary source of C3 and C4 (Lubbers et al. 2017; Ritchie et al. 2004). Previous animal models and clinical studies have revealed that plasma C3 and C4 were increased in patients with acute stroke (Alawieh et al. 2015a; Cervera et al. 2010; Lin et al. 2018). Meanwhile, higher plasma complement levels were associated with an unfavorable outcome, and the predictive value of these markers may depend on the stroke subtype (Stokowska et al. 2011, 2013). In line with previous research results, our analyses also showed that higher C3 was associated with concurrent infection. The relationships had remained significant after adjustment for other risk factors. However, it is difficult to determine the causal relationships among enhancing complement activity, cerebral ischemia injury, and concurrent infection based on the available evidence. We can only preliminarily infer that the complement activity may be closely related to concurrent infection in stroke patients.

Complement factors have different roles within the complement cascade (Carpanini et al. 2019). Studies of human populations have revealed that C3 and C4 are associated with increased levels of cardiovascular risk factors, like obesity, hypertension, and diabetes (Copenhaver et al. 2019; Engstrom et al. 2005; Nilsson et al. 2014). However, high C4 levels may be associated with the incidence of cardiovascular disease, independently of traditional cardiovascular risk factors (Engstrom et al. 2007). Baseline C4 level was an independent predictor of stroke in a broad population of patients referred for coronary angiography (Cavusoglu et al. 2007). These findings are partly consistent with our main results that high plasma C4 levels, but not C3, in the diabetic stroke patients were independently related with concurrent infection and unfavorable outcomes. It is interesting to note that this association was lost in the non-diabetic stroke group.

A recent study with a large sample size by Mellbin et al. showed that not only the artery disorder but also
diabetes per se is associated with complement activation and inflammation (Mellbin et al. 2012). Some of the complement regulatory proteins could be affected by glycation in diabetic stroke (Mellbin et al. 2012). However, the high admission levels of C4 may, at least in part, be a result of the activation of the complement cascade due to acute ischemia (Carpanini et al. 2019). Meanwhile, different parts of the complement system may play different roles in the setting of cardiovascular disease and diabetes (Lau et al. 2019). Although few studies on the C4 and related mechanisms in patients with diabetic stroke, some reasonable deductions have been put forward. Considering that C3 and C4 are expressed and produced in abdominal adipose tissue, cytokines that stimulate the hepatic production may also stimulate the production of lipids and reduce insulin sensitivity (Engstrom et al. 2007). Increased C3 and C4 levels are both associated with metabolic syndrome, including abdominal obesity, independently of inflammatory activity. Moreover, in a Hungarian study, the authors reported a positive correlation between serum C4 levels and BMI, independently of C3 in regression analyses (Phillips et al. 2009). In the diabetic mouse models, a marked association between the increased serum complement activity and the ischemic brain injury

| Table 5 | Selected univariate and multivariate ORs with 95% CIs of association between C3 and C4 levels and NIHSS at discharge |
|---------|----------------------------------------------------------------------------------------------------------------------------------|
| **Diabetic stroke** | **NIHSS at discharge ≤ 10, n** | **NIHSS at discharge > 10, n** | **Univariate OR** | **Multivariate OR** |
| C3       |                                |                                  |                   |
| Lower Third | 32 | 6 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 36 | 3 | 0.44 (0.103–1.924) | 0.40 (0.075–2.163) |
| Upper Third | 33 | 6 | 0.97 (0.283–3.323) | 1.32 (0.31–5.646) |
| C4       |                                |                                  |                   |
| Lower Third | 33 | 4 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 41 | 4 | 0.80 (0.187–3.465) | 2.25 (0.373–13.629) |
| Upper Third | 27 | 7 | 2.14 (0.566–8.084) | 11.26 (1.519–83.488) |
| C3/C4    |                                |                                  |                   |
| Lower Third | 34 | 4 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 31 | 8 | 2.19 (0.601–8.01) | 2.63 (0.526–13.158) |
| Upper Third | 36 | 3 | 0.70 (0.148–3.4) | 0.55 (0.081–3.749) |
| WBC      |                                |                                  |                   |
| Lower Third | 38 | 0 | 1.87 (1.362–2.568) | 2.67 (1.565–4.583) |
| Middle Third | 39 | 2 | 1.0 (reference) | 1.0 (reference) |
| Upper Third | 24 | 13 |                                |                  |
| **Non-diabetic stroke** | **NIHSS at discharge ≤ 10, n** | **NIHSS at discharge > 10, n** | **Univariate OR** | **Multivariate OR** |
| C3       |                                |                                  |                   |
| Lower Third | 93 | 7 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 94 | 13 | 1.83 (0.702–4.81) | 1.84 (0.616–5.506) |
| Upper Third | 85 | 18 | 2.81 (1.12–7.069) | 2.58 (0.867–7.722) |
| C4       |                                |                                  |                   |
| Lower Third | 81 | 9 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 102 | 11 | 0.97 (0.384–2.455) | 1.07 (0.379–3.062) |
| Upper Third | 89 | 18 | 1.82 (0.774–4.28) | 1.76 (0.658–4.753) |
| C3/C4    |                                |                                  |                   |
| Lower Third | 93 | 10 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 85 | 17 | 1.86 (0.807–4.285) | 2.15 (0.83–5.61) |
| Upper Third | 94 | 11 | 1.08 (0.441–2.685) | 1 (0.358–2.795) |
| WBC      |                                |                                  |                   |
| Lower Third | 96 | 7 | 1.0 (reference) | 1.0 (reference) |
| Middle Third | 96 | 7 | 1 (0.338–2.96) | 0.76 (0.226–2.577) |
| Upper Third | 80 | 24 | 4.11 (1.685–10.046) | 3.24 (1.222–8.596) |

Model was also adjusted for: gender, TC, TG, HDL, FBS

Values with significance levels were denoted as follows: * p < 0.05; ** p < 0.01; *** p < 0.001
was demonstrated (Lin et al. 2018). Despite the fact that plasma C3 and C4 are both increased, complement activation in ischemic stroke occurs predominantly by the classic pathway, which involves cleavage of C4 to C4b (Cavusoglu et al. 2007; Pedersen et al. 2009). However, site-targeted inhibition of the alternative complement pathway could modulate post-stroke degenerative and regenerative processes (Alawieh et al. 2015b). The absence of C4 in the alternative pathway may explain why the associations of C4 and concurrent infection and unfavorable outcomes were lost in the non-diabetic stroke group. In a word, C4 is involved in both cerebral ischemia injury and metabolic events and may play a more critical role in the pathogenesis of diabetic stroke (Cavusoglu et al. 2007; Cojocaru et al. 2008). Further studies are needed to explore the mechanism of plasma levels of C4 in the incidence of diabetic stroke.

Our data should be interpreted with some caution due to the limitations of the study. Since the participants in this study were recruited only from one clinical unit, there may have been retrospective bias inherent due to the insufficient sample size. NIHSS was used to assess the patient’s neurologic function, and our study did not explore the relationship between cerebral infarct volume and metabolic brain disease (2021) 36:2313–2322

Table 6 Selected univariate and multivariate ORs with 95% CIs of association between C3 and C4 levels and concurrent infection

| Tertiles          | Concurrent infection | Non-concurrent infection | Univariate OR   | Multivariate OR   |
|-------------------|----------------------|--------------------------|-----------------|-------------------|
| **Diabetic stroke** |                      |                          |                 |                   |
| C3                |                      |                          |                 |                   |
| Lower Third       | 10                   | 28                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 7                    | 32                       | 0.613(0.206–1.823) | 0.603(0.168–2.173) |
| Upper Third       | 17                   | 22                       | 2.164(0.828–5.652) | 6.229(1.674–23.175)** |
| C4                |                      |                          |                 |                   |
| Lower Third       | 9                    | 28                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 12                   | 33                       | 1.131(0.416–3.076) | 2.603(0.721–9.398) |
| Upper Third       | 13                   | 21                       | 1.926(0.694–5.346) | 10.506(2.265–48.733)** |
| C3/C4             |                      |                          |                 |                   |
| Lower Third       | 13                   | 25                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 12                   | 27                       | 0.855(0.329–2.221) | 0.605(0.184–1.991) |
| Upper Third       | 9                    | 30                       | 0.577(0.212–1.571) | 0.315(0.087–1.136) |
| WBC               |                      |                          |                 |                   |
| Lower Third       | 9                    | 29                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 8                    | 33                       | 0.781(0.267–2.289) | 0.918(0.258–3.267) |
| Upper Third       | 17                   | 20                       | 2.739(1.019–7.361) | 4.101(1.214–13.856)** |
| **Non-diabetic stroke** |                  |                          |                 |                   |
| C3                |                      |                          |                 |                   |
| Lower Third       | 81                   | 19                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 82                   | 25                       | 1.3(0.664–2.542) | 1.909(0.892–4.085) |
| Upper Third       | 70                   | 33                       | 2.01(1.05–3.845)** | 3.359(1.546–7.297)** |
| C4                |                      |                          |                 |                   |
| Lower Third       | 70                   | 20                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 89                   | 24                       | 0.944(0.483–1.846) | 1.184(0.566–2.478) |
| Upper Third       | 74                   | 33                       | 1.561(0.819–2.973) | 2.077(0.998–4.325) |
| C3/C4             |                      |                          |                 |                   |
| Lower Third       | 78                   | 25                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 74                   | 28                       | 1.181(0.631–2.208) | 1.375(0.682–2.772) |
| Upper Third       | 81                   | 24                       | 0.924(0.487–1.754) | 0.913(0.45–1.853) |
| WBC               |                      |                          |                 |                   |
| Lower Third       | 89                   | 14                       | 1.0(reference)  | 1.0(reference)    |
| Middle Third      | 74                   | 29                       | 2.491(1.227–5.06)** | 2.267(1.056–4.867)** |
| Upper Third       | 70                   | 34                       | 3.088(1.538–6.198)** | 2.745(1.28–5.883)** |

Model was also adjusted for: gender, TC, TG, HDL, FBS
Values with significance levels were denoted as follows: * p < 0.05; ** p < 0.01; *** p < 0.001
and poor prognosis. If infarct volume per se has a major implication on complement component levels, this may have contributed to our findings of a strong association with outcome. However, as the results have shown, we did not find a strong correlation between NIHSS score at admission and plasma complement levels in the acute phase, which spoke against any major effect of infarct size on these parameters. In addition, DM types were not distinguished. However, considering that people with type 1 DM usually show symptoms in early life and all of our patients are middle-aged adults, all cases of DM in this study are very likely to be type 2 DM (Li et al. 2017). Further studies should be performed to expand the sample size and investigate the relevant immune pathways.

**Conclusion**

In summary, compared with non-diabetic stroke, our data showed that high plasma C4 level at admission was associated with unfavorable clinical outcomes in diabetic stroke, independently of traditional risk factors. Our results may help stratify the ischemic stroke patients not only according to stroke severity but also the importance of controlling for stroke etiology.

**Acknowledgements** We would like to thank Prof. Xingshun Xu for his valuable comments on this manuscript.

**Authors’ contributions** Ximeng Zhang, Yi Yang, and Jianqiang Ni designed the study, Ximeng Zhang, Jun Yin, Kai Shao, Wei Liu, Yiqing Wang, Shanshan Diao, Shicun Huang, and Qun Xue evaluated the subjects and collected the data. Le Yang and Yi Yang analyzed the data. Ximeng Zhang, Jun Yin, and Kai Shao wrote the initial draft, with Jianqiang Ni and Yi Yang participating in revising this manuscript.

**Funding** This work was supported by the grants from The Natural Science Foundation of the Jiangsu Higher Education Institutions of China (20KJB320021), National Key R&D Program of China (2017YFC0110304), and Provincial Key R & D projects of Jiangsu (BE2019666).

**Data availability** Original data of the present study are available from the corresponding author upon reasonable request.

**Declarations**

**Ethics approval and consent to participate** This study involving human participants was reviewed and approved by the Institutional Review Board of The First Affiliated Hospital of Soochow University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. The experiments comply with the current laws of the country in which they were performed. All patients gave informed consent.

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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