High Serum Complement Component C4 as An Unique Predictor of Unfavorable Outcomes in Diabetic Stroke

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Abstract

Objective: Previous studies demonstrated that diabetic stroke patient had a poor prognosis and excess activation of the complement system in the peripheral blood. In this study, the association of serum complement levels with prognosis of diabetic stroke was examined.

Methods: Patients with acute ischemic stroke were recruited and were divided into two groups according to the 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).

Results: A total of 426 patients with acute ischemic stroke (116 diabetic stroke and 310 non-diabetic stroke) were recruited in this study. There were significant differences on hypertension, CHD, TG, HDL, FGB, C4, and mortality rate between two groups. Furthermore, the values of complement protein levels were divided into tertiles. In diabetic stroke group, serum C4 level at acute phase in the upper third was independently associated with NIHSS score at discharge and concurrent infection. This associations were not significant in non-diabetic stroke.

Conclusion: High serum C4 level at admission, as an unique significant predictor, was associated with unfavorable clinical outcome in the diabetic stroke, independently of traditional risk factors.

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 serious for diabetic patients compared with 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 a 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 the pathophysiology of both stroke and diabetes, the specific inflammatory processes involved may differ between them(Bao et al. 2018).In diabetes mellitus, islet β-cell dysfunction and obesity-driven insulin resistance induced by inflammation in adipose tissue are involved in the pathogenesis(Saltiel and Olefsky 2017). Meanwhile, other inflammation-related mechanisms like endothelial dysfunction, atherosclerosis and increased plaque vulnerability play important roles in cerebrovascular disease, especially for ischemic stroke(Roth et al. 2018; Ruparelia et al. 2017).

The complement system, as a major inflammatory mediator, seems to play a dual role in stroke(Ma et al. 2019).Products in the process of complement activation can protect neurons against excitotoxicity-induced death and contribute to healing(Alawieh et al. 2015).On the other hand, in stroke animal models, inhibition of complement activation reduced infarct volume and neurological impairments(Alawieh et al. 2015; Ma et al. 2019; Szeplaki et al. 2009). In humans, elevated plasma levels of C3 and C5b-9 were

positively correlated with the unfavorable clinic outcome(Mocco et al. 2006; Szeplaki et al. 2009). Compared with vascular disease, the relationship between complement system and diabetes mellitus may be closer(Mellbin et al. 2012).Plasma levels of C3 is an more effective and more highly specific 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 for 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 patients in a proportion of one-sixth. Routine blood and biochemical tests, ECG, and a baseline brain CT/MRI scan were performed in all patients at admission, laboratory investigations for vascular risk factors, duplex sonography of the carotid and vertebral arteries, and a thorough cardiac investigation were all taken. Diabetes was defined as 1) self-reported DM; 2) using of diabetes medication; 3) fasting whole blood glucose ≥ 7.0 mmol/L and/or random whole blood glucose ≥ 11.1 mmol/L; 4) patients according to national or local patient registers with diabetes.

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℃) and at least one other typical symptoms (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 hours of admission). Only 426 patients with AIS (116 diabetic stroke and 310 non-diabetic stroke) were included and formed the basis of this report (Figure 1). All patients received the same medical advice and treatment according to the current guidelines, including 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 an overnight fasting. Complement C3 and C4 were measured using Beckman Specific Protein (America, model: IMMAGE800). Reagents bought in Beckman Coulter (USA) co. LTD (Immune scattering turbidimetry). All test reagents are within the validity period and the quality of the instrument is under control. 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 severity of 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). The secondary outcome was 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 differences among categorical variables were assessed by the Chi-square test. 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 priori power analysis was conducted to calculate sample size. Considering the correlation between smoking status and gender in China, we did not bring them into the adjustment factors at the same time. 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 result(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.

Table 1
The characteristics of patients with AIS

Characteristics	Patients (N = 426)
Demographics and medical history	
Age in years, median (IQR)	68 (57, 75)
Male, n (%)	299 (70.20)
Smoking, n (%)	174(40.80)
Diabetes, n (%)	116 (27.20)
Hypertension, n (%)	301 (70.70)
Atrial fibrillation, n (%)	48 (11.30)
Coronary disease, n (%)	17 (4.00)
Hyperlipidemia, n (%)	10 (2.30)
Clinical features	
FGB in mmol/l, median (IQR)	5.17 (4.62, 6.55)
C3 in g/L, median (IQR)	0.87 (0.76, 0.99)
C4 in g/L, median (IQR)	0.21 (0.18, 0.25)
C3/C4, median (IQR)	4.06 (3.50, 4.64)
WBC in $\times 10^9$ /L, median (IQR)	6.92 (5.66, 8.64)
L in $\times 10^9$ /L, median (IQR)	1.52 (1.11, 1.98)
N in $\times 10^9$ /L, median (IQR)	4.61 (3.48, 6.12)
NLR, median (IQR)	2.81 (4.46, 2.07)
TC in mmol/L, mean ± SD	4.20 ± 1.09
TG in mmol/L, median (IQR)	1.29 (0.96, 1.69)
HDL in mmol/L, median (IQR)	0.93 (0.78, 1.12)
LDL in mmol/L, median (IQR)	2.52 (1.86, 3.17)
NIHSS at admission, median (IQR)	3.00 (1.00, 7.00)
Hospitalization days, median (IQR)	14 (11.00, 17.00)

Abbreviations: IQR, interquartile range; FGB, fasting blood glucose; 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.

Characteristics	Patients (N = 426)
Outcomes	
NIHSS at discharge, median (IQR)	1.50 (0.00, 6.00)
Concurrent infection, n (%)	111 (26.10)
Mortality, n (%)	9 (2.10)

Abbreviations: IQR, interquartile range; FGB, fasting blood glucose; 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.

Comparison of demographic and clinical characteristics

Depending on whether the patient was diabetic or not, the participants were divided into two groups: the diabetic stroke group with 116 patients and non-diabetic strokes group with 310 patients. Statistical analysis indicated that there were significant differences on hypertension, CHD, TG, HDL, FGB, C4, and mortality rate between two groups ($p < 0.05$). However, there was no difference on 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, and other factors between two groups ($p > 0.05$, Table 2).

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
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
FGB 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 ×109 /L, median (IQR)	7.17 (5.87, 8.65)	6.82 (5.57, 8.66)	0.360
L in $\times 10^9$ /L, median (IQR)	1.45 (1.10, 1.90)	1.58 (1.11, 1.99)	0.445
N in $\times 10^9$ /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

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).

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**	-

Note: * $p < 0.05$; ** $p < 0.01$.

C4 was associated with poor outcome 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 very small, no useful separate analysis could be made.

Table 4
Cutoffs for tertiles of plasma C3 and C4 levels

	Diabetic stroke	Non-diabetic stroke
C3, g/L		
Lower Third	< 0.83	< 0.79
Middle Third	0.83–0.94	0.79–0.95
Upper Third	> 0.94	> 0.95
C4 g/L		
Lower Third	< 0.20	< 0.19
Middle Third	0.20–0.25	0.19–0.23
Upper Third	> 0.25	> 0.23
C3/C4		
Lower Third	< 3.68	< 3.76
Middle Third	3.68–4.23	3.76–4.42
Upper Third	> 4.23	> 4.42
WBC, in $\times 10^9$ /L		
Lower Third	< 6.32	< 6.08
Middle Third	6.32–8.21	6.08–7.86
Upper Third	> 8.21	> 7.86

Table 5
Selected univariate and multivariate ORs with 95% CIs of association between C3 and C4 levels and NIHSS at discharge

Tertiles	NIHSS at discharge ≤ 10, n	NIHSS at discharge > 10, n	Univariate OR	Multivariate OR
Diabetic stroke				
C3				
Lower Third	32	6	1.0(reference)	1.0(reference)
Middle Third	36	3	0.444(0.103–1.924)	0.402(0.075–2.163)
Upper Third	33	6	0.97(0.283–3.323)	1.324(0.31–5.646)
C4				
Lower Third	33	4	1.0(reference)	1.0(reference)
Middle Third	41	4	0.805(0.187–3.465)	2.255(0.373–13.629)
Upper Third	27	7	2.139(0.566–8.084)	11.262(1.519–83.488)*
C3/C4				
Lower Third	34	4	1.0(reference)	1.0(reference)
Middle Third	31	8	2.194(0.601–8.01)	2.632(0.526–13.158)
Upper Third	36	3	0.708(0.148-3.4)	0.552(0.081–3.749)
WBC				
Lower Third	38	0	1.87(1.362–2.568)***	2.678(1.565–4.583)***
Middle Third	39	2		
Upper Third	24	13		
Non-diabetic stroke				

Note: These factors were also adjusted in the multivariate regression analysis: gender, TC, TG, HDL, FGB. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Tertiles	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	1.837(0.702–4.811)	1.841(0.616–5.506)
Upper Third	85	18	2.813(1.12–7.069)*	2.588(0.867–7.722)
C4				
Lower Third	81	9	1.0(reference)	1.0(reference)
Middle Third	102	11	0.971(0.384–2.455)	1.077(0.379–3.062)
Upper Third	89	18	1.82(0.774–4.28)	1.768(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)	2.158(0.83–5.61)
Upper Third	94	11	1.088(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)	0.764(0.226–2.577)
Upper Third	80	24	4.114(1.685–10.046)**	3.241(1.222–8.596)*

Note: These factors were also adjusted in the multivariate regression analysis: gender, TC, TG, HDL, FGB. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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				

Note: These factors were also adjusted in the multivariate regression analysis: gender, TC, TG, HDL, FGB. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Tertiles	Concurrent infection	Non-concurrent infection	Univariate OR	Multivariate OR
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)**

Note: These factors were also adjusted in the multivariate regression analysis: gender, TC, TG, HDL, FGB. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

For 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; $p < 0.05$). Model were established for group of confounding factors: gender, TC, TG, HDL, FGB. Interestingly, this association was lost in 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, FGB; adjusted OR, 10.506; 95%CI, 2.265–48.733; $p < 0.01$). This association was also lost in 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).

Discussion

In our study, the plasma levels of C3 and C4 between diabetic stroke and non-diabetic strokes 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 patient, 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.

Complement, as an important branch of the innate immune system, 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 some 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 up regulated by inflammatory signals(Carpanini et al. 2019; Lubbers et al. 2017). 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 main 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. 2015; 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 stroke subtype(Stokowska et al. 2011; Stokowska et al. 2013). In line with previous research results, our analyses also showed 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 relationship among enhancing complement activity, cerebral ischemia injury and concurrent infection 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 their 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 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 there were 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 is expressed and produced in abdominal adipose tissue, cytokines that stimulate the hepatic production of them may also stimulate the production of lipids and reduce insulin sensitivity(Engstrom et al. 2007). Increased C3 and C4 levels are both associated with the metabolic syndrome including abdominal obesity, independently of inflammatory activity. Moreover, in a Hungarian study, they 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 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). In a word, C4 is involved in both cerebral ischemia injury and metabolic events and may play a more important role in the pathogenesis of diabetic stroke(Cavusoglu et al. 2007; Cojocaru et al. 2008). However, 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 limitations of the study. Since the participants in this study were recruited only from one clinical unit, there may have 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 poor prognosis. If infarct volume per se has a major implication on complement components levels, this may contribute to our findings of a strong association with outcome. However, as results had shown, we did not find 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

Results from a number of studies point out that patients with diabetic stroke may have a poor prognosis, and the exaggerated activation of the complement system could be one of the reasons. In summary, compared with non-diabetic stroke, our data showed that high plasma C4 level at admission was associated with unfavorable clinical outcome in the 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.

Declarations

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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 the manuscript.

Conflict of interest.

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

Ethics approval and consent to participate

This study involving human participants were 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.

Availability of data and materials

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

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Figures

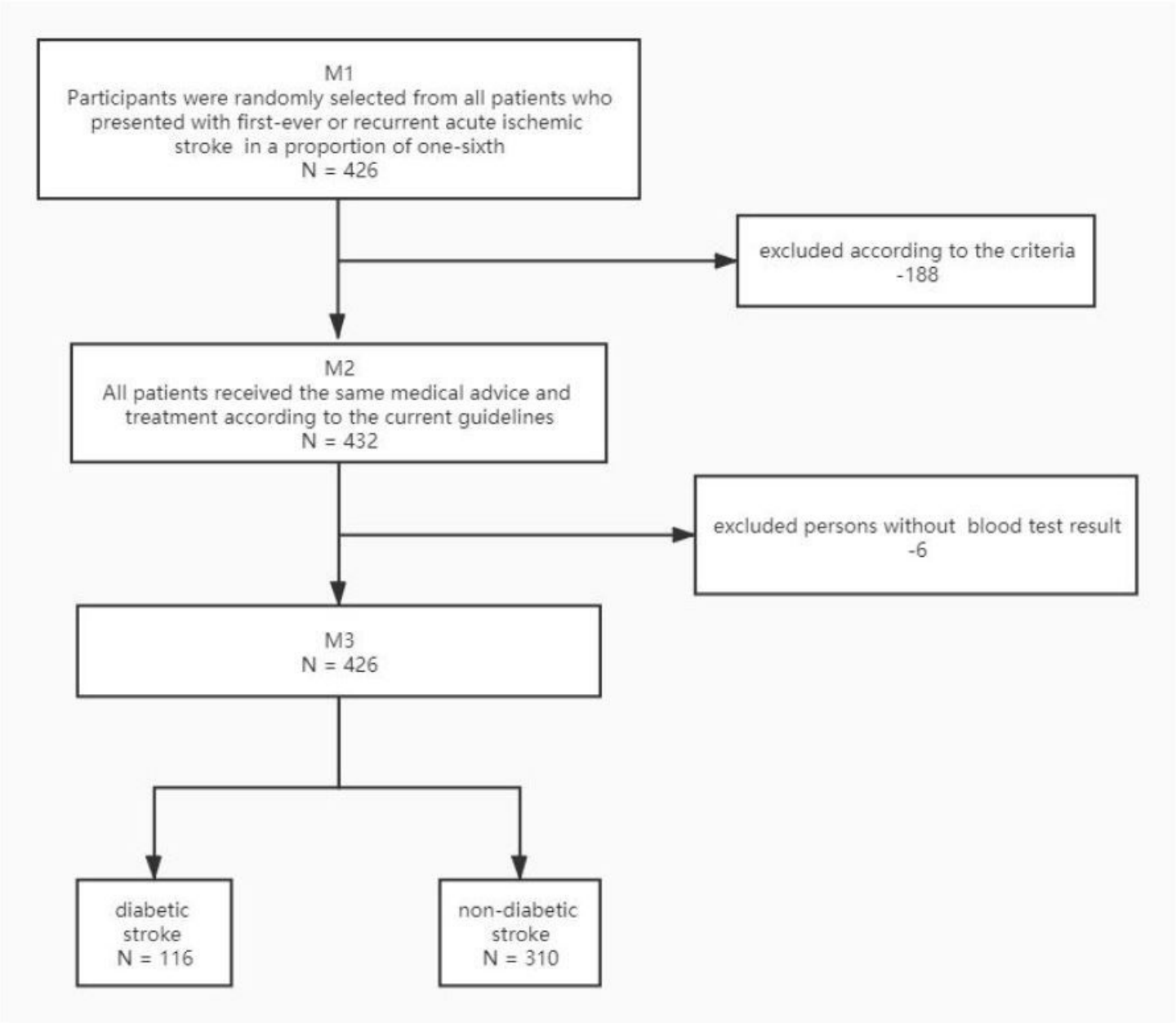

Figure 1

Flow diagram of participants' selection