Circulating immunocytes and complements are correlated with severity of ischemic stroke

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Research

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Abstract

Background

Immune factors are found to be involved in the pathophysiological process of ischemic stroke. However, the clinical role of the immune system in stroke remains unclear. Our study was designed to investigate the correlation between immunocytes (T cell, B cell, NK cell), complements and stroke severity.

Methods

236 patients with first-ever ischemic stroke were included in our study and divided to mild, moderate and severe groups according to NIHSS score at stroke onset. Modified Rankin Scale (mRS) was used to assess short-term prognosis 3 months after stroke. We also collected clinical data and test circulating T cells, CD8 + T cells, CD4 + T cells, B cells, NK cells, C3, C4, complement factor B (CFB) of each patient.

Results

We found NK cell ($p = 0.015$), CFB ($p = 0.007$), C3 ($p = 0.035$) were significantly elevated in groups with higher NIHSS score. CD4 + T cell ($p = 0.009$), T cell ($p = 0.001$) were decreased in groups with higher NIHSS score. In multivariate logistic regression, CFB and C3 were independently associated with severity of stroke. CD4 + T cell, NK cell, T cell were independently associated with severe ischemic stroke. NK cell ($p = 0.049$), CFB ($p = 0.035$), C4 ($p = 0.028$) were significantly higher in groups with higher mRS score. In binary analyses, CFB ($p = 0.009$, OR: 1.056, 95%CI: 1.014–1.099), C4 ($p = 0.011$, OR: 134.444, 95%CI: 3.115–5803.504) were independently associated with prognosis at 3 months after stroke adjusting onset.

Conclusions

Elevated circulating CFB was correlated with both severe symptom at stroke onset and worse prognosis. It indicates that CFB may be a predictor of stroke severity and prognosis. Reduced T cell counts, elevated C3 and NK cell were associated with severe symptom at stroke onset. C4 was associated with short-term prognosis.

1. Background

Ischemic stroke is one of the leading causes of disability and mortality worldwide, and its incidence is increasing[1]. Recent years, inflammation has been shown to play an important role in the pathophysiology of ischemic stroke[2, 3]. Progression of symptomatic intracranial large vessel disease is also associated with a proinflammatory state[4]. Following an acute focal brain injury during stroke, neural cell death orchestrates a secondary immune response characterized by glial activation, recruitment of peripheral immune cells, and release of cytokines and chemokines[3]. While post-stroke inflammation

may contribute to the clearance of tissue debris and tissue repair, most published literature indicates that inflammation in the brain during the acute phase of stroke promotes the expansion of stroke lesions and worsens neurodeficits[5, 6].

Several studies using animal models suggests that immune cells such as lymphocytes, NKT cells, NK cells may be recruited several hours after stroke onset[7, 8]. Amount of reports suggest that T lymphocytes play very crucial roles in the tissue damage following cerebral ischemia[9, 10]. However, whether T cells exacerbate or alleviate the symptoms of stroke remains controversial[11, 12]. Higher NK cells counts was reported to be associated with post-stroke infection[13]. Some studies report that immunosuppression after stroke may cause infection and aggravate patients situation. Complement system were also found to play important roles in acute ischemic stroke[14]. However, what roles complement system and immunocytes played in the progression of ischemic stroke is still unclear.

To date, there are limited data about the effects of lymphocytes and complements on cerebral stroke severity and prognosis. Accordingly, the purpose of the present study was to access the association of plasma complements, T cell, NK cell and cerebral stroke severity in clinical cases.

2. Subjects And Methods

Study Population

236 patients were involved from the First Affiliated Hospital of Soochow University during March 2018 to March 2019. Participants were randomly selected from all patients in a proportion of one-fourth. All the participants were diagnosed as first-ever acute ischemic stroke according to World Health Organization recommendations.

The initial sample included 280 patients. The exclusion criteria were (1) patients were younger than 18 years old; (2) patients with systemic infection; (3) patients with autoimmune disease such as systemic lupus erythematosus, rheumatism, etc; (4) cerebral infarction caused by vasculitis, subarachnoid hemorrhage, sinus venous thrombosis, or severe head trauma; (5) patients took immunosuppressant, corticosteroids in three months. 236 patients were involved and formed the basis of this report (Fig. 1). All patients received treatment according to the current guidelines.

Clinical information collection

Demographic data (age, sex), history of conventional vascular risk factors (hypertension, diabetes mellitus) were obtained. Neuroimage (CT or MR) were performed in all patients at admission. Stroke severity was evaluated by the National Institutes of Health and Stroke Scale (NIHSS) at admission. The modified Rankin Scale (mRS) was finished 3 months after stroke onset to evaluate short-term prognosis. This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University. All participants or their relatives were informed of the study protocol, and their written informed consents were obtained before included.

Blood Sampling and test

Fasting blood was collected via venipuncture in BD Vacutainer® (NJ, USA) tubes at 7:00 am on the morning after admission. Blood samples were centrifuged at 4000r/min for 10min. Immunoturbidimetry (Beckman Specific Protein, model: IMAGE800) was used to test T cells (CD3+), T-helper cells (CD3 + CD4+), T cytotoxic cells (CD3 + CD8+), B cells (CD19+), natural killer (NK) cells (CD3-CD56 + CD16+), CFB (complement factor B), C3, C4.

Statistical Analysis

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. Due to a predominantly non-Gaussian distribution of data, nonparametric tests were employed for the statistical analysis. Differences in characteristics among each group were examined with the chi-square test for proportions for categorical variables and the Mann-Whitney U test or Kruskal-Wallis test for continuous variables. Bonferroni test was used in post-hoc analysis of multivariate comparison. Multivariate logistic regression was used to analyze the association of immune factors and stroke severity. Binary logistic regression was used to analyze the association of immune factors and prognosis. Significance for the results was set at $p < 0.05$, two tails. Statistical analysis was performed in SPSS 25.0.

3. Results

Demographics and characteristics of participants

A total of 236 patients with acute ischemic stroke were included in our study. Baseline characteristics were shown in Table 1. The median age of all patients was 69 (58, 75) years old. 158 (66.95%) patients were male and the ratio of male to female was about 7:3. 173 (73.31%) patients had hypertension. 55 (23.31%) patients had diabetes. NIHSS score was 1.0 (1.0, 6.0). MRS score was 1.0 (0, 2.0).

Table 1
The characteristics of all included patients

Characteristics	Patients (N = 236)
Demographics and medical history	
Age in years, median (IQR)	69 (58, 75)
Male, n (%)	158 (66.95)
Diabetes, n (%)	116 (27.20)
Hypertension, n (%)	173 (73.31)
Clinical features	
NK cell (%), median (IQR)	14.30 (9.53, 22.85)
CFB (mg/dL), median (IQR)	35.90(30.45, 40.88)
C3 (g/L), median (IQR)	0.87 (0.75, 0.97)
CD4 + T cell (%), median (IQR)	41.89 (35.80, 49.19)
T cell (%), median (IQR)	70.45 (62.21, 75.63)
CD4+/CD8+, median (IQR)	1.76(1.29, 2.44)
C4 (g/L), median (IQR)	0.21 (0.18, 0.25)
CD8 + T cell (%), median (IQR)	24.30 (19.16, 29.55)
B cell(%), median (IQR)	12.22(8.57, 16.64)
Outcomes	
NIHSS, median (IQR)	1.5 (0.0, 6.0)
MRS, median (IQR)	1.0(0, 2.0)

Immunocytes, complements and stroke severity

Baseline characteristics

All the patients were categorized as mild (NIHSS score 0–4), moderate (NIHSS score 5–10), and severe group (NIHSS score ≥ 11) according to NIHSS score at admission[15]. The characteristics of the patients are presented in Table 2. Gender ($p = 0.176$), prevalence of hypertension ($p = 0.510$) and diabetes ($p = 0.831$) showed no significant difference in three groups. Age showed significant difference between severe group and other two groups. The patients in severe group are older than mild group ($p = 0.004$) and moderate group ($p = 0.026$). Age is not significantly different between mild group and moderate group ($p = 0.539$).

Table 2

Comparison of demographic, clinical characteristics, immunocytes and complements among patients with different level of NHISS score

NHISS	0–4	5–10	≥ 11	P value	Post-hoc#
Demographics and medical history					
Number	161	57	18	-	-
Gender (male%)	70.19	63.16	50	0.176	1 = 2 = 3
Age (year)	68 (57, 75)	70 (55, 75)	76 (69, 84)	0.004**	1 = 2 < 3
Hypertenstion(%)	71.43	75.44	83.33	0.510	1 = 2 = 3
Diabetes(%)	23.6	21.05	27.78	0.831	1 = 2 = 3
Immunocytes and complements					
NK cell (%)	13.69 (9.35, 21.32)	14.25 (9.35, 21.32)	24.65 (14.00, 31.71)	0.015*	1 = 2 < 3
CFB (mg/dL)	34.50 (29.75, 40.20)	37.50 (32.15, 44.45)	39.30 (33.35, 47.80)	0.007**	1 < 2 = 3
C3 (g/L)	0.85 (0.75 0.95)	0.89 (0.75, 1.03)	0.90 (0.85, 1.04)	0.035*	1 < 2 = 3
CD4 + T cell (%)	42.38 (36.22, 49.78)	44.13 (36.55, 49.23)	36.13 (31.93, 40.04)	0.009**	1 = 2 > 3
T cell (%)	71.12 (63.55, 76.52)	71.33 (62.87, 75.02)	57.78 (50.85, 63.87)	0.001**	1 = 2 > 3
CD4+/CD8+	1.72 (1.31 2.50)	1.73 (1.26 2.40)	1.98 (1.44, 2.61)	0.556	1 = 2 = 3
C4 (g/L)	0.21 (0.17, 0.24)	0.23 (0.18, 0.27)	0.23 (0.20, 0.28)	0.037*	1 = 2 = 3
CD8 + T cell (%)	24.52 (20.28, 29.97)	23.88 (19.35, 29.13)	17.85 (11.56, 26.18)	0.031*	1 = 2 = 3
B cell (%)	11.60 (8.15, 15.57)	13.72 (9.75, 17.27)	14.89 (8.54, 18.39)	0.100	1 = 2 = 3
*P < 0.05					
**P < 0.01					
# In the column of post-hoc analysis, 1 = mild group (NHISS 0–4), 2 = moderate group (NHISS 5–10), 3 = severe group (NHISS ≥ 11)					

Comparison of immunocytes and complements

Table 2 shows comparison of each immune factors in three groups. Statistical analysis indicated that NK cell ($p = 0.015$), CFB ($p = 0.007$), C3 ($p = 0.035$) were significantly elevated in groups with higher NHISS score. CD4 + T cell ($p = 0.009$), T cell ($p = 0.001$) are decreased in groups with higher NHISS score. There was no significant difference on C4, CD8 + T cell and B cell. Results of post-hoc analysis was list in Table 2.

Association of immunocytes, complements and stroke severity

In multivariate logistic regression (Table 3), CFB, C3 were independently associated with severity of stroke adjusting for age, gender, hypertension, diabetes. CD4 + T cell, NK cell, T cell were independently associated with severe ischemic stroke.

Table 3
Association of immunocytes, complements and stroke severity
(multivariate logistic regression)

	NHISS	OR	P value
CFB	0–4	1 (reference)#	-
	5–10	1.057 (1.017–1.097)	0.000**
	≥ 11	1.090 (1.026–1.157)	0.005**
C3	0–4	1 (reference)	-
	5–10	6.802 (1.103–41.951)	0.039*
	≥ 11	17.388 (1.072–282.064)	0.005**
NK cell	0–4	1 (reference)	-
	5–10	0.999 (0.968–1.030)	0.948
	≥ 11	1.053 (1.007–1.101)	0.023*
CD4 + T cell	0–4	1(reference)	-
	5–10	1.001 (0.968–1.035)	0.974
	≥ 11	0.920 (0.863–0.979)	0.009**
T cell	0–4	1(reference)	-
	5–10	0.989 (0.959–1.019)	0.466
	≥ 11	0.909 (0.865–0.955)	0.000**
#Mild group as reference in multivariate logistic regression			

Immunocytes, complements and short-term prognosis

Baseline characteristics

All the patients were categorized as mild (mRS 0–2) and severe group (mRS > 2) according to mRS score at 3 months after stroke onset. The characteristics of the patients are presented in Table 4. Gender ($p = 0.086$), prevalence of hypertension ($p = 0.573$) and diabetes ($p = 0.993$) showed no significant difference in three groups. The patients in severe group are significantly older than mild group ($p = 0.003$).

Table 4
Comparison of demographic, clinical characteristics, immunocytes and complements among patients with different level of mRS score

MRS	0–2	> 2	P value
Demographics and medical history			
Number	193	43	-
Gender (male%)	69.43	55.81	0.086
Age	68 (56, 75)	73 (66, 79)	0.003**
Hypertenstion(%)	72.54	76.74	0.573
Diabetes	23.32	23.26	0.993
Immunocytes and complements			
NK cell (%)	13.69 (9.21, 22.20)	17.22 (12.78, 25.50)	0.049*
CFB (mg/dL)	35.50 (30.30, 40.60)	37.80 (33.50, 45.30)	0.035*
C3 (g/L)	0.86 (0.75, 0.96)	0.88 (0.79, 1.06)	0.091
CD4 + T cell (%)	42.81 (36.19, 49.60)	38.68 (33.96, 47.44)	0.128
T cell (%)	71.25 (62.93, 76.10)	66.79 (58.51, 74.78)	0.050
CD4+/CD8+	1.75 (1.33, 2.50)	1.81 (1.21, 2.35)	0.875
C4 (g/L)	0.21 (0.18, 0.25)	0.23 (0.19, 0.28)	0.028*
CD8 + T cell (%)	24.40 (19.29, 29.59)	23.53 (17.65, 29.35)	0.799
B cell (%)	11.96 (8.46, 15.99)	13.63 (8.91, 17.15)	0.495
*P < 0.05			
**P < 0.01			

Comparison of immunocytes and complements

Table 4 shows comparison of each immune factors in two groups according to mRS score. NK cell ($p = 0.049$), CFB ($p = 0.035$), C4 ($p = 0.028$) are were significantly elevated in groups with higher mRS score.

Association of immunocytes, complements and short-term prognosis

In binary analyses, CFB ($p = 0.009$, OR: 1.056, 95%CI: 1.014–1.099), C4 ($p = 0.011$, OR: 134.444, 95%CI: 3.115-5803.504) were independently associated with prognosis at 3 months after acute ischemic stroke adjusting for age, gender, hypertension, diabetes(Table 5). NK cell was not independently associated with short-term prognosis.

Table 5
Association of immunocytes, complements and prognosis at 3 months (binary logistic regression)

	OR	P value
CFB	1.056 (1.014–1.099)	0.009**
C4	134.444 (3.115-5803.504)	0.011*
NK cell	1.007 (0.975–1.040)	0.687
*P < 0.05		
**P < 0.01		

4. Discussion

It is known that the central nervous system actively interacts with the immune system, both directly and via intermediates[16]. First damage of ischemic stroke is caused by occlusion of artery and necrosis of neurons. Meanwhile, immune cells play critical roles in secondary damage after stroke onset[17]. A lot of studies elucidate that NK cells, lymphocytes, C3 and C4 and other immune factors participate in the development and destabilization of atherosclerotic plaques[18–20]. T cells and NK cells were also found to recruit and infiltrate in the ischemic hemisphere[21, 22, 20]. Some studies proposed that ischemic stroke can induce immune suppression which may cause infection [23]. However, some other research found that immune cells aggravate the situation of infarct[12]. Inhibiting immune system activity could attenuate ischemic brain injury.

Our study found that elevated circulating CFB, C3 were independently associated with severity of stroke at onset. CD4 + T cell, NK cell, T cell were independently associated with severe ischemic stroke. CFB, C4 were independently associated with prognosis at 3 months.

The complement system is an essential part of innate immunity, typically conferring protection via eliminating pathogens and accumulating debris. Previous studies found that the level of C3 in plasma was higher in ischemic stroke patients than that in the healthy controls [24–26]. Elevated C3 in plasma from embolic ischemic stroke or cryptogenic stroke patients was associated with worse neurological

outcomes 3 months and 2 years after ischemia onset[27, 28, 7]. Complement inhibition could improve the outcomes of ischemic stroke in many animal models[29]. There are limited clinical studies about stroke and C4 or CFB.

The complement system consists of three different activation pathways: classic, lectin, and alternative. All three pathways merge at the level of complement component C3 activation. The complement component C4 plays a central role in the activation of the classic and lectin pathways. CFB is an activator of C3 and plays roles in alternative pathway. Our study shows elevated CFB is associated with worse neurological deficits both at stroke onset and 3 months after acute ischemia. It suggests that alternative pathway may play an important role in secondary damage of stroke. Complement system is hyperactive in stroke process, especially in severe cases. CFB may be a good predictor of stroke severity. According to our study, elevated C3 is associated with severe symptom in early stage of stroke. Elevated C4 is associated with worse short-term prognosis. Combined previous studies, activation of complement system is correlated with severity and worse prognosis of stroke.

NK cells are part of the innate immune system. They control CNS inflammation by killing proinflammatory microglial cells, which are activated within minutes of ischemia onset[30]. Sylvie De Raedt[31] found the number of circulating NK cells within the first hour after stroke was elevated, especially in patients with pneumonia later. Lünemann[30] found that NK cells catalyzed neuronal death and determined the infarct size. Our study suggest that elevated circulating NK cells are associated with severe stroke. Combined above studies, we suggest that elevated circulating NK cells count may be a potential indicator of severe stroke.

T lymphocytes are part of the adaptive immune system and are divided to several subtypes depending on their functional properties[12]. Previous studies found that stroke induced a dramatic and immediate loss of T-lymphocytes, which contributed to the stroke-induced immunosuppression. Immunosuppression can induce susceptibility to infection, which may aggravate the symptom and prognosis of stroke. There are also study reporting that CD4 + T cell loss may emerge as a predictive marker for post-stroke infection allowing and early identification of patients at risk[11]. Combined above studies, we suggest that loss of T cell and CD4 + T cell may be potential indicators of severe stroke.

As we know, complements, T cells, NK cells, B cells are all parts of immune system. In our study, we found NK cells, complements were positively correlated with severity and prognosis of stroke. However, T cells were negatively correlated with severity and prognosis of stroke. The results reveal the complexity of the role of the immune system in the pathogenesis of ischemic stroke. Immunocyte and immune factors play different roles in the course of stroke, which need more studies to discover.

In our study, we also found that age was correlated with severity and prognosis of stroke in the analysis of baseline characteristics. Older patients have a poor prognosis, which is consistent with our common clinical knowledge.

Some study limitations should be considered. Since our series is from a single medical center, small study population might have introduced statistical error. We test immune cells and complements, which cannot elucidate the immune mechanisms of stroke comprehensively. Further inflammatory cytokines and animal models are expected. More studies are needed to elucidate the molecular and cellular mechanisms of how complement components exert their functions in different stages of ischemic stroke to optimize the intervention of targeting the complement system.

5. Conclusion

Stroke triggers a robust and sustained shift in systemic immunity in patients. Our study found that elevated circulating CFB was correlated with both severe symptom at stroke onset and worse prognosis. It indicates that CFB may be a predictor of stroke severity and prognosis. Reduced T cell counts, elevated C3 and NK cell were associated with severe symptom at stroke onset. C4 was associated with short-term prognosis.

Abbreviations

CFB

Complement factor B

NIHSS

National Institutes of Health and Stroke Scale

mRS

Modified Rankin Scale

NK cell

Natural killer cell

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital of Soochow University. Informed consent was signed by each participant.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interest

The authors declare that they have no competing interests.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Ni Jianqiang and Zhang Ximeng designed the study. Huang Shicun, Xu Jialiang, Xiao Xinyi, Lu Haifeng, and Wang Yiqin evaluated the subjects and collected the data. Zhang Sheng and Xu Jialiang finish the test of blood sample. Zhang Ximeng and Yang Yi analyzed the data. Zhang Ximeng and Huang Shicun wrote the initial draft, with Ni Jianqiang, Yang Yi and Xue Qun participating in revising the manuscript. All authors contributed to the article and approved the submitted version.

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Figures

Figure 1

Flow diagram of participants selection.