**Title**:Association of peripheral blood immunophenotype indicators with prognostic survival in patients with acute ischemic stroke

Association of peripheral blood immunophenotypic markers with prognostic survival in patients with acute ischemic stroke

Peripheral blood immunophenotype indicators for prognostic survival in acute ischemic stroke

Selecting the influence of optimal peripheral blood immunophenotypic indicators for prognostic survival in acute ischemic stroke

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**Abstract**

**Background:** Whether the prognosis of acute ischemic stroke is affected by peripheral blood immunophenotype indicators is remain unclear. Thus, our study aimed to investigate the relationship between peripheral blood immunophenotype indicators and prognostic survival in patients with acute ischemic stroke.

**Methods:** This retrospective study was made up of 255 individuals who diagnosed the acute ischemic stroke and 147 individuals who diagnosed the other mild neurological disease but not vascular diseases from 2016 and 2019. Peripheral blood was collected within 24 hours after admission, and immunophenotype indicators analysis was performed by flow cytometry. Kaplan-Meier survival analysis was used to analyze the survival curves of the control group and the acute ischemic stroke group. Besides, univariable and multivariable Cox were used to analyze associations between different cell subtypes and acute ischemic stroke during the 5-year follow-up period. Stepwise regression was used to select important immunophenotype indicators related to survival. Besides, according to gender groups, one-way ANOVA was used to find the indicators with no gender difference. Univariable and multivariable regression analyses were used to identify independent predictive factors of acute ischemic stroke. (AIS)

**Results:** A total of 500 patients assessed for eligibility who included 300 individuals who diagnosed the acute ischemic stroke and 200 individuals who diagnosed the other mild neurological disease but not vascular diseases. Because of basic information and laboratory indicators missing partly, a total of 402 individuals completed the analysis of flow immunophenotype and basic characteristics. Mean age was 64.2±18.4 years, males represented 71.3% of the population in the acute ischemic stroke. Univariable and multivariable regression analyses identified that there were seven immunophenotype indicators that may be become independent clinical prognostic marker significantly which the influence of gender on immunophenotype indicators were excluded. These indicators respectively were the percentage of Cytotoxic T cells, absolute number of total Treg cells, absolute number of memory B cells, absolute number of total monocytes, absolute number of non-classical monocytes, absolute number of CD56high NK cells and absolute number of CD16+ NK cells. Absolute number of total Treg cells , absolute number of memory B cells and absolute number of CD16+ NK cells were significantly associated with decreased risks of OS in acute ischemic stroke group, hazard ratio and confidence interval respectively were HR: 0.97, 95% CI:0.95-0.99, *p* <0.05; HR:0.90, 95% CI:0.85-0.95, *p* <0.05; HR:0.93, 95% CI:0.88-0.98, *p* <0.05. However, the percentage of Cytotoxic T cells, absolute number of total monocytes, absolute number of non-classical monocytes and absolute number of CD56high NK cells were significantly associated with increased risks of OS in acute ischemic stroke group, hazard ratio and confidence interval respectively were HR:1.18, 95% CI: 1.03-1.33, *p* < 0.05; HR: 1.13, 95% CI: 1.05-1.21, *p* < 0.05; HR: 1.09, 95% CI: 1.02-1.16, *p* < 0.05; HR: 1.13, 95% CI: 1.05-1.21, *p* < 0.05.

**Conclusions:** The present study identified that immunophenotype biomarkers in peripheral blood may have great clinical significance in the acute ischemic stroke. Predictors of early acute stroke outcome may contribute to stroke treatment. It may be of great significance to seek peripheral blood immunophenotype biomarkers for clinical prognosis in patients with acute ischemic stroke.

**Keywords** acute ischemic stroke, immunophenotype, prognostic survival.

**Trial registration number:** ChiCTR2000040207

**Introduction**

Cerebral vascular disease including cerebral ischemic stroke remains a major cause of long-term disability and death worldwide. Ischemic stroke accounts for approximately 80% of stroke incidences1, which is a leading cause of death and physical disability word-wide. However, treatments that effectively limit the tissue injury and brain dysfunction are still elusive, and the current therapeutic approaches for the acute treatment only rely on blood flow restoration by thrombus lysis or mechanical removal2, treatments that effectively limit the tissue injury and brain dysfunction are still elusive. Prediction of functional outcome after ischemic stroke is crucial for patients and clinicians for allocation of healthcare resources and optimization of patient care.13Stroke produces profound local and systemic immune responses that engage all major innate and adaptive immune compartments.4 However, whether immunophenotype indicators influence the prognosis of acute ischemic stroke remains unclear. Over the past years, inflammatory response after cerebral ischemia has risen broad interests5. Poststroke immune regulation including accumulation of microglia and infiltration of the ischemic hemisphere by macrophages, lymphocytes, dendritic cells (DCs) and neutrophilic flux. The extent of neuronal damage seems to correlate with the degree of innate immune activity and numerous studies have demonstrated the critical role of the cellular and humoral immune system in postischemic brain injury6. Therefore, as a pivotal role in cerebral vascular disease, immune system requires extensive studies.

Circulating neutrophils predominate in humans (50–70%), while in rodents, circulating leukocytes mainly consist of lymphocytes (75–90%). Besides potential differences between humans and animals, poststroke neuroinflammation and the efficacy of anti-inflammatory treatments differ substantially among commonly used animal stroke models suggesting that this heterogeneity contributes to the translational failure. Altogether, single studies analyzing the post-ischemic immune cell infiltration revealed inconclusive results.

In experimental animal models such as mice and rat, the crucial functions of invading immune cells and proinflammatory cytokines have been well 7-10investigated7-10, while the whole immune reaction pictures in human ischemic stroke is barely unknown. Even the poststroke immune regulation were mainly focused on local lesion, such as resident immune cells and cytokines, whereas, the activation in peripheral blood circulation were limited. Given the differences of systemic blood and immune responses in between animal models and human, it poses a challenge how to transform discoveries in animal models into ‘druggable’ mechanisms of ischemic stroke. Therefore, little is known on the reaction of systemic immune response after ischemic stroke, and is still controversial whether the immune response has benefits or hazards.

Here we used immunophenotyping assays by single-cell mass cytometry to comprehensively and functionally characterize the systemic immune response within 24 hours after ischemic stroke in peripheral blood samples from 255 patients. We investigated the relationship between overall survival and immunophenotyping of peripheral blood, which included lymphocyte, monocyte, DC cell and NK cell subsets. In addition, the survival rate were followed to assess the relationship between immune response and prognosis. After ischemic stroke the systemic immune response were inhibited, a factor that led to poor prognosis and lower overall survival rate. These set of immunophenotyping with peripheral blood provide a promising and easily accessible post-stroke biomarkers to predicts functional outcomes after ischemic stroke.

**Patients and Methods**

**Study Design and Population**

A retrospective study was conducted on a primary cohort of the patients who diagnosed as acute ischemic stroke and other mild neurological diseases but not vascular diseases between January 2016 and December 2019 in Zhejiang Provincial People’s Hospital, Hangzhou, Zhejiang Province, China. The patients were eligible for enrollment if they had ischemic stroke, and then were evaluated by stroke specialists. A total of 500 patients assessed for eligibility that included 300 individuals were diagnosed with acute ischemic stroke, were regarded as the acute ischemic group, another 200 individuals were diagnosed with other mild neurological diseases but not related to any vascular diseases, were regarded as the ‘control group’, including **i**) 78 with myasthenia gravis; **ii**) 60 with dizziness; **iii**) 32 with peripheral neuropathy; **iv**) 30 with Parkinson’s disease. 53 patients were excluded with lack of complete basic information or laboratory indicators, then by immunophenotyping assays using single-cell mass cytometry, 402 patients who included 255 patients with the acute ischemic stroke group and 147 patients with the control group which including **i**) 73 with myasthenia gravis; **ii**) 40 with dizziness; **iii**) 17 with peripheral neuropathy; **iv**) 17 with Parkinson’s disease totally. (**Figure 1**)This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee of Zhejiang Provincial People’s Hospital. All the authors vouch for the validity of the data and adherence to the protocol.

**Baseline characteristics and laboratory measurements**

The baseline characteristics of the participants included age, sex, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), the history of hypertension, smoking, alcohol, and the history with antihypertension drugs and lipid-lowering drugs. Height and weight were measured with the participants standing without shoes or heavy outer garments. BMI was calculated by dividing weight in kilograms by height in meters squared.

The samples of the peripheral blood were collected within 24 hours after admission. Laboratory variables included red blood cell, white blood cell, platelet count, hemoglobin, hematocrit, and mean corpuscular volume, were measured on a Coulter Counter STKS sample testing system (Coulter Corp, America) according to standard procedures provided by Sysmex Corporation (Japan). Total protein (TP), albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose (GLU), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride, serum creatinine levels were measured using standard protocols provided by Beckman-Coulter Experimental System Corporation (America).The above data were collected and reviewed retrospectively.

**Flow Cytometry**

Within 24 hours after admission, the peripheral blood samples in patients with diagnosed acute ischemic stroke were collected, anticoagulated with heparin and stained. Each sample was divided into five tubes, antibodies were added to each flow tube in turn according to the detection scheme, and then 100 microliters of peripheral blood were taken and incubated in the absence of light for 15 minutes, then 1× ammonium chloride lysate (0.15M NH4CL, 10mM NaHCO3, 1mM EDTANa2) was fully mixed and not exposed to light for 10 minutes, and finally 500g centrifugation for 5 minutes, then the supernatant was removed, and the samples were re-suspended and mixed with 200 microliters of PBS, and detected on the computer. More detailed trial procedures were described in the reference.11 The detection scheme was shown in **Table S1**.

**Statistical analysis**

**Basic Statistics**

The continuous variables were summarized as, means and standard deviations, or medians and interquartile ranges, and the categorical variables were expressed as frequencies and percentages. Continuous variables were compared using the Student’s *t* test and categorical variables were compared using the chi-square test, as appropriate. The difference of immunophenotypes indicators was analyzed by one-way ANOVA. Considering the influence of gender on confounding factors, we first removed the immunophenotypes indicators which were caused by gender, and then determine the difference between the control group and the acute ischemic stroke. Pearson correlation coefficient was used to measure the correlation between immunophenotypes indicators.

**Variable Selection**

As described herein, 22 difference indicators variables were entered into the selection process. Least absolute shrinkage and selection operator (LASSO) regression was applied to minimize the potential collinearity of variables measured from the same patient and over-fitting of variables. We used L1-penalized least absolute shrinkage and selection regression for multivariable analyses, augmented with 10-fold cross validation for internal validation. This is a Cox regression model that penalizes the absolute size of the coefficients of a regression model based on the value of λ. With larger penalties, the estimates of weaker factors shrink toward zero, so that only the strongest predictors remain in the model. The most predictive covariates were selected by the minimum λ. The R package “glmnet” statistical software (R Foundation) was used to perform the LASSO regression.

**Survival analysis**

Kaplan-Meier survival analysis was used to analyze the survival curves of the control group and the acute ischemic stroke group. Univariate Cox survival analysis of differences between and within groups was performed by log-rank test. LASSO regression was used to select important immunophenotype indicators related to survival based on cross validation for internal validation, then established multi-factor analysis and multivariate survival model was performed to calculate hazard ratio (HR) and confidence interval (CI). “Survival” and “Survminer” R package packages were mainly used in the survival analysis.

**Receiver Operating Characteristic Curve**

The areas under the receiving operating characteristic (ROC) curves were used to evaluate the selection accuracy of immunophenotype indicator in identifying acute ischemic stroke patients with survival risk. The area under the ROC curve (AUC), true positive rate (also called sensitivity or recall), and false positive rate (specificity) are represented in a graphical plot. According to the best cut-off value of ROC curve, the importance of selection indicators were set as high and low groups. Kaplan-Meier survival analysis was used to analyze the survival curves of the high and low groups. “survivalROC” R packages were mainly used in the ROC curve analysis.

In this paper, all tests were two-tailed, with a significant *p*-value threshold was defined as 0.05. Statistical analysis were conducted in R statistical software( version 3.6.1; http://www.Rproject.org).

**Results**

**characteristics of the patients and immunophenotype indicator**

The basic characteristics of the patients were shown in **Table 1**. The mean age of the acute ischemic stroke group and the control group (mild neurological disease but not related to any vascular diseases) was 64.2 ±18.4 years and 58.3±14.5 years respectively(*p*=0.11). BMI was 23.8 ± 4.1 and 22.8 ± 5.2 respectively (*p*>0.05). Of them, 182(71.3%) and 69 (46.9%) were males in the acute ischemic stroke group and the control group, respectively ( *p*<0.05). The differences immunophenotype indicators caused by gender are shown in **Table S2**. Unilateral difference analysis was used to find 42 indicators with no difference regarding gender in our study, as was shown in **Table 2**, 22 immunophenotype indicators were found to difference between the ischemic stroke group and the control group. The 22 immunophenotype indicators correlation coefficient matrix is shown in **Figure 2.**

As shown in the **Table 3**, The acute ischemic stroke group had higher concentration of ALT, AST, GLU, TBIL, DBIL, IBIL, CREA and ALP compared with the control group, which were statistically significant (*p*<0.05). The acute ischemic stroke patients had lower concentration of PLT, LDL-C, HDL-C, TC, ALB and triglyceride compare with the control group which was statistically significant (*p*<0.05). However, all these laboratory indicators were within the normal reference range. In addition, the *p*-value of RBC, WBC, Hemoglobin, Hematocrit, MCV and TP were more than 0.05, with no statistically significant.

**Comparison of survival outcomes**

Follow-up information was available for acute ischemic stroke and the control group whose were followed up to 5 years. The probability of overall survival of patients with the acute ischemic stroke was significantly lower than that of the control group, which had [distinct](javascript:;)ly statistical significance [log-rank test, p<0.0001] as was shown in **Figure 2**. However, the analysis of gender between the two groups showed a no significant difference. [log-rank test, p=0.7] (**S-figure1**).

**Feature Selection of immunophenotype indicator**

Of immunophenotype indicator feature, 22 features were reduced to 7 potential predictors on the basis of 251 patients in the acute ischemic stroke cohort (**Figure 3 A and 3B**), and were features with nonzero coefficients in the LASSO Cox regression model. Including the percentage of Cytotoxic T cells, absolute number of total Treg cells, absolute number of memory B cells, absolute number of total monocytes, absolute number of non-classical monocytes, absolute number of CD56high NK cells and absolute number of CD16+ NK cells. (**Figure 3****C)**

**Univariate and multivariable Cox** **regression analysis**

The results of univariate analysis are shown in Figure 4 and showed that immunophenotype indicator including the percentage of Cytotoxic T cells, absolute number of total monocytes, absolute number of CD16+ NK cells, absolute number of non-classical monocytes and absolute number of CD56high NK cells were contribute to increase the survival risk [Hazard ratio>1, *p*<0.05]. The results of multivariate analysis are shown in Figure 5. The results showed that there were statistically significant differences in 7 immunophenotype indicator(*p*<0.05). These were contribute to increase the survival risk included the percentage of Cytotoxic T cells [HR:1.18;95%CI:1.03-1.33], absolute number of total monocytes [HR:1.13;95%CI:1.05-1.32], absolute number of non-classical monocytes [HR:1.09;95%CI:1.02-1.16] and absolute number of CD16+ NK cells [HR:1.13;95%CI:1.05-1.21].

**Evaluate the selection accuracy of immunophenotype indicator**

The examined 7 immunophenotype indicator were able to identify with good accuracy of prediction in survival risk for acute ischemic stroke patients (**Figure 6)**. The AUC for the percentage of Cytotoxic T cells was 0.846 [95% CI, 0.781 to 0.854; best cut-off 333.95], Absolute number of total Treg cells was 0.843 [95% CI, 0.774 to 0.894; best cut-off 382.38], Absolute number of non-classical cells was 0.854 [95% CI, 0.801 to 0.914; best cut-off 421.26], Absolute number of Memory B cells was 0.879 [95% CI, 0.834 to 0.934; best cut-off 192.49], Absolute number of total monocytes cells was 0.820 [95% CI, 0.734 to 0.854; best cut-off 706.37], absolute number of CD56high NK cells was 0.912 [95% CI, 0844 to 0.954; best cut-off 603.37] and absolute number of CD16+ NK cells was 0.72[95% CI, 0844 to 0.954; best cut-off 13.37]. The analysis of 7 immunophenotype indicator between the two groups based on best cut-off showed a probability of overall survival is significant difference. [log-rank test, *p*<0.05] (**S-figure2**).

**Discussion**

It is well known that there is not only an immediate pro-inflammatory response to stroke, but also post-stroke immunosuppression responses.12,13,14,15,16 In this study, we used a single-cell mass cytometry approach to analyze a large number of immunophenotypes indicators in peripheral blood with the systemic immune response within 24 hours after the acute ischemic stroke. 54 immunophenotypes indicators were statistically significant, especially T cells, monocytes, DC cells and Treg cells, indicated that a systemic immune response was inhibited significantly with the acute ischemic stroke after acute onset. In addition, inhibited immune response leading to poor survival in long-term significantly. More importantly, no one has comprehensively and systematically studied the relationship between peripheral blood immunophenotype indicators and the prognosis of acute ischemic stroke. This study aimed to discuss the importance of immunophenotype indicators which help doctors monitor patient outcomes in the acute ischemic stroke.

Many stroke patients experience severe consequences, however, reliably predicting stroke outcome remains challenging. Several attempts to identify characteristics associated with poor outcomes have been made. Almost 40% of stroke patients have a poor outcome at three months after the index event. Predictors for stroke outcome in the early acute phase may contribute to stroke treatment.17 The prognostic value of peripheral blood biomarkers in patients with the acute ischemic stroke could potentially be of great importance for clinical routine.

In our experiment, we found that 16 immunophenotypes indicators were relevant with the prognosis of acute ischemic stroke for univariate survival analysis (log-rank test, p<0.05). Besides, the relationship between different immunophenotypes indicators and survival probability were compared using multivariate survival analysis. 13 significant immunophenotypes indicators were selected by stepwise regression method. Peripheral blood of immunophenotypes indicators provide a promising and easily accessible biological substrate to search for such biomarkers and lay foundation on immunophenotypes and prognosis and survival after stroke.

Previous studies mainly focused on in humans18,19 and rodent models20 of the local and short-term immune response to stroke. However, the most studies are focused of animal models, it is hard to predict the situations in human. In contrast, from a clinical perspective, targeting inflammation in human may not be easy, especially for the long-term observations. Therefore, we proposed that using cytometry to analyze and compare the whole immunophenotypes indicators after acute ischemic stroke is possible and effective.

21-2425-2728,2930,313233,34Several lines of evidence suggested that immune responses detectable in peripheral blood relate to early innate and later adaptive immune responses in the brain.21-24 In our study, we focused on a large of immunophenotypes indicators of peripheral blood within 24 hours after acute ischemic stroke. More importantly, we found a strong correlation between immunophenotypes and survival probability of the acute ischemic stroke. For example, by calculating the HR of each immunophenotype indicator, as shown in **Figure 3**, 8 immunophenotypes indicators could decrease the survival probability and 8 immunophenotypes indicators could increase the survival probability in the acute ischemic stroke group. Besides, it was seen that the percentage of non-classical monocyte was the most significant immunophenotypes indicators with the survival probability. The absolute number of Th2 cell was the most significant indicators to increase the survival probability.

By analyzing immunophenotypes indicators of the peripheral blood in our studies, compared with the two groups of total T cell, we concluded that the acute ischemic stroke of it was lower than the control group. The percentage of and absolute number of total monocyte, non-classical monocyte and classical monocyte of the acute ischemic group were higher than the control group in our study.

Monocytes are a type of leukocytes that play an important role in the post-ischemic inflammation.35 Although only about 5% of white blood cells in peripheral blood are monocytes, these cells, as an important part of the vascular innate immune system, can produce large amounts of inflammatory cytokines.36 Hongbing Liu et al suggested that monocyte-to-high-density lipoprotein ratio (MHR) may be a significant and independent predictor of unfavorable functional outcome in patients with acute ischemic stroke.37 Besides, previous findings suggested that number of monocytes and plasma MCP-1 level could be clinical prognostic biomarkers as early predictors of disease outcome in patients with ischemic stroke.38 Monocytosis may also be an independent risk factor for myocardial infarction and cerebral ischemic disease and the monocyte count is also correlated with the National Institutes of Health Stroke Scale (NIHSS) score in patients with AIS.39,40 Xiaoyu Dong et al indicated that a peripheral monocyte count ≥0.53 × 109/L is an independent prognostic marker on 90-days in patients with acute ischemic stroke treated with rtPA thrombolysis.41 However, their study only examined peripheral blood monocytes, whereas we examined immunophenotype of all monocytes. In their study demonstrated that patients in the poor outcome group had a significantly higher monocyte count than those in the good outcome group. Consequently, it’s consistent with our results that indicated our research is more scientific and authentic.

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Furthermore, T cells may also affect various non-immunological pathways by interacting with brain-resident immune cells and modulating mechanisms such as neurogenesis and angiogenesis43.Therefore, the whole amount of T cells are crucial. In this study, we found both the percentage and absolute number of total T cells are significantly lower (*p*<0.001) after acute ischemic stroke than the control group. In addition, among all the ischemic stroke patients, lower total T cells also leading to lower survival rate, suggesting that T cells are involved in the progression and prognosis of stroke.

Regulatory T cells (Tregs) are a subpopulation of T cells that represents 5-10% of circulating CD4+T cells.44 Tregs that control inflammatory and immune responses in the body, are closely related to the pathogenesis of ischemic stroke. Tregs are crucially involved in the modulation of basal immunity, maintenance of immune homeostasis, and regulation of immune response to diseases.45 It was reported that after experimental stroke, the total number of peripheral blood T cells was significantly reduced, while the proportion of Tregs was significantly increased.46 Their results are consistent with ours. The proportion of Tregs in the peripheral blood was dramatically downregulated on day 1 after tMCAO, returning to normal amount on day 3 and to about 10% on day 7, which indicates the redistribution of Tregs post-stroke. It is speculated that Tregs migrate from the periphery to the brain in the early stage of stroke and exert early effects on cerebral ischemia through the peripheral immune system.47,48 49,50Besides, animal studies revealed that Tregs played a protective role in experimental stroke.51 Previous reports suggested that endogenous Tregs are neuro-protective and that boosting the number of circulating Tregs robustly protects against ischemic brain injury.46,52,53

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Dendritic cell (DC) is a type of cell involved in innate and adaptive immunity. In clinical studies, numbers of circulating DCs are inversely correlated with clinical stage and ischemic infarct size. 56 In agreement with the clinical studies, murine stroke models have also shown a strong correlation between brain parenchymal DCs and infarct volumes.57 Using flow cytometry, Gelderblom M, Leypoldt F, Steinbach K, et al have shown that DCs comprise a large portion of all infiltrating immune cells.58 Importantly, many studies suggest that DC amplification after cerebral ischemia exacerbates stroke outcomes.56 DCs present in the infarct zone could stimulate and activate T cells, induce a long-lasting immune response, and worsen stroke outcome. In addition, a transient decrease of DCs in the circulation might contribute to stroke-induced immunodepression.56

However, there are still limitations in this study, including that it was a retrospective study, and the immunophenotypes and cytokines such as IFN-γ in the peripheral blood should be analyzed continuously to better show the correlation with the prognosis. In addition, this study was limited to 1 hospital site, and limited sample size, which might restrict the generalizability of the results.59

In conclusion, our study lays the foundation for more comprehensive human studies in the future aimed at targeting specific markers and immunophenotype of peripheral blood. In addition, it was testified that answer the question of whether stroke-related phenotypical and functional immunological changes could be tracked in peripheral blood. The results demonstrated the utility of a deep immune profiling approach with single-cell mass cytometry to comprehensively and functionally characterize the systemic immune response within 24 hours after ischemic stroke in peripheral blood samples. Predictors of early acute stroke outcome may contribute to stroke treatment. The prognostic value of peripheral blood biomarkers in patients with the acute ischemic stroke may be of great significance to clinical routine.

**Declaration of interests**

The authors declare no competing financial interests.

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**Author contributions**

YW, Guo-Bo Chen, XMT conceived the study and designed the analysis, YW and KL curated the clinical data and the patients followed-up by telephone, Yong-Ran Cheng and Guo-Bo Chen performed [statistic](javascript:;)al [analysis](javascript:;), WMN conducted flow immunophenotype analysis. KL, Yong-Ran Cheng, WY, and Guo-Bo Chen wrote the first draft of the manuscript, and all other authors contributed to revision of the manuscript.

**Additional information**

Supplementary tables and figures can be found at the website of the journal.

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**Abbreviations**

**CI, confidence interval; HR, hazard ratio; MV, multivariable; NS, not**

**significant; OS, overall survival; SD, standard deviation.**

**Figure legends**

**Figure 1 Flowchart of the study.**

**Figure 2 Prognostic incidence of overall survival curve comparisons between patients with the acute ischemic stroke and the control group**

**Figure 3 Typical immunophenotypes indicators of** Univariate Cox survival analysis conditions in the acute ischemic stroke

**Figure 4 Effects of high and low immunophenotypes indicators (According to the mean value) on survival and prognosis in the acute ischemic stroke.**

**Figure S1 Distribution of immunophenotypes indicators difference between the control group and the acute ischemic group.**

**Figure S2** **54 immunophenotypes indicators of clinical Statistical significance by** Univariate Cox survival **survival conditions in the acute ischemic stroke group.**

**Figure S3 16 immunophenotypes indicators between the acute ischemic stroke and the control group.**

**Figure S4 Effects of high and low 54 immunophenotypes indicators of clinical Statistical significance by** Univariate Cox survival **survival conditions in the acute ischemic stroke group.**

**Figure 5 Multivariate Cox survival analysis in the ischemic stroke**

**Table 1 Characteristics of study participants**

**Table 2 Characteristics of immunophenotype between patients with the acute ischemic stroke and the control group**

**Table S1 Detection scheme for eight-color antibody panels proposed by the Human Immunophenotyping Consortium**

1. Gr?Schel K, Schnaudigel S, Edelmann F, et al. Growth-differentiation factor-15 and functional outcome after acute ischemic stroke. Journal of Neurology 2012;259:1574-9.

**Figure 2**

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