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The role of eosinophils in stroke: a pilot study

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Abstract. – OBJECTIVE: To evaluate the factors that may have an impact on the prognosis of ischemic stroke.

PATIENTS AND METHODS: The study included 973 patients who were diagnosed with an ischemic stroke. The patients were divided into 6 groups according their eosinophil counts' level and occurred times. All patients were supervised by NIHSS score in three months.

RESULTS: Baseline characteristics of all study groups were comparable. Thirty-four patients in group 2 and group 5 had higher eosinophil counts. In addition, the patients experienced impaired function on the face, but no impairment of limbs. All patients in these groups recovered quicker than the other groups ($p < 0.05$). Furthermore, 169 patients in both group 3 and group 6 had lower eosinophil counts. These patients experienced functional impairment in limbs and difficulty recovering from the disease. The NIHSS score was lower in both group 2 and group 5, compared with group 3 and group 6 ($p < 0.05$).

CONCLUSIONS: This study suggests that eosinophil counts may have a significant impact on outcomes in stroke patients. The data underscore the importance in further investigating eosinophil dysregulation as well as the potential relationship between eosinophil dysregulation and organ functions in stroke patients.

Key Words:
Eosinophil, Stroke, Platelet, Outcome.

Introduction

Eosinophils are known to have bioactive roles in immunity and are capable of storing preformed cytokines, chemokines, and growth factors that can be released rapidly in response to immunoregulatory and inflammatory cytokines such as IL-4, IL-6, and IL-10[1]. Moreover, recent studies suggest that eosinophils play an important role in thrombosis[2-5]. However, mechanisms underlying this role are poorly understood.

We analyzed data of 973 stroke patients who were administered into our hospital. The first purpose of this study was to evaluate whether there was a dysregulation of the eosinophil in stroke patients. The second purpose was to determine whether eosinophil count can predict the outcome of stroke patients.

Patients and Methods

Study Population

A study was performed on a cohort of ischemic stroke patients who were admitted to the neurological department from October 1, 2012 to October 1, 2014 and received a standard thrombolytic therapy.

This study was approved by Siping Central Hospital of China Medical University Review Board. All patients in the cohort were 18 years of age or older, and were admitted to the intensive care unit (ICU) either directly or transferred from the emergency floor after a prior period of hospitalization that required a sub-acute level of care. "Acute" was defined as those patients who were diagnosed within 24 hours. Patients were excluded from this study if (1) experienced severe cerebral hemorrhage, (2) failed to follow up because of life-threatening conditions, (3) had missing stratification information, or (4) without standard care and rehabilitation. This exclusion criterion was chosen to ensure that all patients were treated according to the best medical practice. Those patients, whose main clinical problems were not stroke, were also excluded.

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For the data analysis, 973 patients were categorized into six groups. Group 1 consisted of 399 patients (233 male; average age = 61.7 ± 3.7) who were recently diagnosed and had normal eosinophil counts. Group 2 consisted of 13 patients (10 male; average age = 63.9 ± 5.1) who were newly diagnosed and had higher eosinophil counts. Group 3 consisted of 90 patients (58 male; average age = 63.1 ± 4.6) who were recently diagnosed and had lower eosinophils count. Group 4 consisted of 371 patients (205 male; average age = 72.1 ± 8.6) who reoccurred and had normal eosinophil counts. Group 5 consisted of 21 patients (17 male; average age = 70.8 ± 7.9) who reoccurred and had higher eosinophil counts. Group 6 consisted of 79 patients (34 male; average age = 71.4 ± 5.8) who reoccurred and had lower eosinophil counts. The general characteristics of the patients were shown in Table I. Blood was taken from the internal jugular veins.

Definition of Ischemic Stroke

Stroke was diagnosed based on the World Health Organization (WHO) criteria (rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than of vascular origin)[6]. Although a continuous sign for 24 hours is necessary for stroke diagnosis, the patients were treated as soon as the stroke was indicated to avoid unnecessary delay of therapy.

Laboratory Measurements

Serum levels of total cholesterol (TC), and triglyceride (TG) were measured by an enzymatic colorimetric method using the autoanalyzer Hitachi 911 (Boehringer Mannheim, Indianapolis, IN, USA). Blood cells were analyzed by an automated blood cell counter LH-750 (Beckman Coulter, Brea, CA, USA).

Treatment Protocol

The following treatment protocol was used: (1) anti-platelet aggregation such as ozagrel (Changchun Extrawell Pharmaceutical Co., LTD. Changchun, Jilin, China), and aspirin (Bayer Co., LTD., China); (2) anti-oxidative stress (Edaravone Injection, Jilin Boda Pharmaceutical Co., LTD. Liaoyun, Jilin, China); (3) medications for improving cerebral circulation (Shuxuetong Injection), Mudanjiang Youbo Pharmaceutical Co., LTD. Mudanjiang, Liaoning, China); (4) cerebroprotein hydrolysate (Yunnan Mensheng Pharmaceutical Co., LTD. Kunming, Yunnan, China); (5) rehabilitation.

Data Collection

Data were collected through one-on-one interviews using standardized scripts. Each respondent provided a short non-technical description of limbs' function scale and health status.

Outcomes

The primary outcome included the length of stay in the ICU, readmission to the ICU, infection, neurological dysfunction, eosinophil and platelet counts, and length of stay in the hospital. A p-value of < 0.05 was considered statistically significant. Clinical outcome was followed up at four weeks (by phone) and three months (in person).

The percentage of time that a patient's eosinophil count remained within one of the two levels (i.e. $< 0.02 \times 10^9$/L or $> 0.5 \times 10^9$/L) was evaluated. This value was used based on a modi-

Table I. General characteristics of stroke patients.

Variables	G1	G2	G3	G4	G5	G6
Age Range	45-81	43-79	45-74	43-79	44-83	45-78
mean±SD	61.7±3.7	63.9±5.1	63.1±4.6	72.1±8.6	70.8±7.9	71.4±5.8
Males/females	233/166	10/3	58/32	213/158	17/4	34/45
Hypertension	217	7	48	266	11	4
Diabetes mellitus	196	3	21	173	6	19
Infection	46	6	36	64	9	28
Current smoking	196	7	42	183	14	43
Current drinking	76	5	27	93	9	31
Total cholesterol, mg/dl	5.32±0.69	5.46±0.32	5.52±0.45	5.39±0.66	5.41±0.47	5.48±0.63
Triglycerides, mg/dl	2.16±1.03	2.21±0.98	2.31±1.15	2.33±0.96	2.41±0.89	2.38±0.76

G:group.

fication of the time-weighted limbs' function analysis. The median values of each eosinophil level were used for statistical analysis.

Statistical Analysis

Data were presented as means ± standard error of the mean (SEM) unless otherwise noted. A p value of less than 0.05 was considered significant. Student's t-tests were used to compare continuous variables between the groups. Chi-square and Fisher's exact tests were used to compare proportional data between the groups. The multivariate logistic analysis was performed to adjust for covariates and parameter estimates, and likelihood ratio tests were generated. Interactions of the effects between variables were examined. Statistical analyses were performed with SPSS19.0 (IBM SPSS Company, Chicago, IL, USA).

Results

General Characteristics of the Stroke Patients

Data from a total of 973 patients were included in the analysis. The general characteristics of these patients were shown in Table I. Of these, 591 patients (60.7%) had a documented history of hypertension, 418 patients (42.9%) of diabetes mellitus, and 179 patients (18.4%) of infection. All patients were subdivided based on eosinophil count and recurrence of ischemic stroke. There was no significant difference between recent onset and recurrent patients.

Multiple Factors Predict Outcomes

Table II shows a comparison of unadjusted outcomes in recent onset and recurrent patients. The data demonstrate that three-month outcomes were better in patients who had higher eosinophil counts as compared with those who had normal or lower eosinophil counts. In addition, patients who had higher eosinophil counts, as compared with normal and lower eosinophil counts, showed better limb function in both recent onsets and recurrent patients. Furthermore, in both recent onset and recurrent patients, the NIHSS score was significantly lower. The recovery was quicker in patients who had higher eosinophil counts as compared with those who had normal or lower eosinophil counts (5.3 ± 0.6 *vs.* 18.6 ± 1.2 in new occurred patients 6.1 ± 0.4 *vs.* 17.1 ± 0.5 in reoccurred patients, respectively, $p = 0.01$). Furthermore, more patients who had lower eosinophil counts experienced hypertension.

The data also demonstrate that diabetes and hypertension were factors that predict the patients' outcomes. Readmission rates to the ICU, and length of stay (LOS) in the ICU or hospital were significantly lower in G2 and G5 (Table II).

The Relationship Between Eosinophils Count and Platelet Parameter on the Ischemic Stroke Patients' Outcomes

Our data also showed, in both recent onset and recurrent patients, that the mean eosinophil count in G2 was 12.5% and 26.6% higher than the G1 and G3 groups respectively. In addition, the mean hospital LOS in G2 group was shorter than

Table II. Outcome data of the total population (Ggroup).

Variables	G1	G2	G3	G4	G5	G6
Readmission rate to ICUa (%)	76/399	1/13*#	41/90	32/371	3/21*#	48/79
ICU LOS (days)	3.4±1.4	0.6±0.8*#	6.2±0.9	4.1±0.8	0.8±0.4*#	6.8±1.1
Hospital LOS(days)	19.8±1.4	8.2±2.3*#	23.4±1.8	20.4±2.1	7.3±1.9*#	22.6±2.2
NIHSS values during 24h treatment, % readings	15.6±0.6	6.4±1.7*#	21.1±0.9	16.4±0.3	6.9±0.8*#	19.6±1.1
NIHSS values before left ICU treatment, % readings	13.4±1.2	5.8±0.9*#	19.3±0.7	13.0±0.9	6.4±0.6*#	17.4±0.4
NIHSS values during 4 weeks treatment, % readings	11.4±1.2	5.3±0.6*#	18.6±1.2	12.1±1.3	6.1±0.4*#	17.1±0.5
NIHSS values during 3 months treatment, % readings	10.6±0.9	4.9±0.3*#	16.7±0.8	11.9±1.1	5.8±0.6*#	16.9±0.5

[a]Readmission rate defined as at least one readmission per patient.
LOS: Length of stay (days)
*p: compared to G1 and G4, *$p < 0.05$; #p: compared to G3 and G6, #$p < 0.05$

Table III. The relationship of eosinophil count levels and impaired part in ischemic stroke patients.

Variables	G1	G2	G3	G4	G5	G6
Face	173	13	11	156	21	16
Limb	332	0	90	309	0	79

G1 and G3 groups (52.6% and 73.9% respectively). These values were expressed as mean ± SEM for descriptive purposes. The relationship between eosinophil counts and organ functional impairment in the ischemic stroke patients are shown in Table III. The data showed that patients with higher eosinophil counts displayed impaired function on the face with no functional impairment in the limbs. However, patients with lower eosinophil counts had impaired function in the limbs and difficulty recovering from the stroke. The impaired limb functions were improved followed by an eosinophil counts increase in five patients, who had lower eosinophil counts in both the new and recurring stroke groups (G3 and G6). However, the eosinophil counts did not increase over normal, and their impaired limb function was still worse, even the limb function was better than the other patients in G3 and G6.

Next, we determined which factors, either eosinophil count or platelet distribution width (PDW), is related to the outcomes in recent onset ischemic stroke patients. Table IV showed that patients who had higher eosinophil count did not show any malfunction in their limbs ($p < 0.05$). In addition, those patients who had higher eosinophil count showed lower PDW (shown in Table IV). There were two patients who had lower eosinophil count and malfunction in their limbs at the beginning. However, their limb function recovered when the eosinophil counts increased.

A forward stepwise regression was performed to identify covariates that predicted NIHSS (Table V). The base model derived by logistic regression identified eosinophil count, PDW as predictors of NIHSS scores. There was an apparent association between increased NIHSS scores in recurrent patients with hypertension in three months.

Discussion

In the current study, we reported our analysis of factors that might predict the outcome of stroke patients. We showed that stroke patients, who had higher eosinophil counts, displayed functional impairment only on the face but not in limbs. Stroke patients, who had lower eosinophil counts, showed functional impairment in the limbs and had difficulty recovering from the disease. To our knowledge, this is the first report that eosinophil counts can be an independent prediction factors for the outcome of stroke patients.

Our data suggest that circulating eosinophils might contribute to the recovery of stroke[7]. In the current study, we observed that stroke patients who had lower eosinophil counts experienced a malfunction in the limbs and had difficulty recovering from the disease (shown in Table III). More interestingly, when the eosinophil counts increased, the patients' limb function improved accordingly. Therefore, it appears that eosinophils may play a protective role in endothelial function. Since eosinophils produce numerous growth factors such as vascular endothelial growth factors (VEGF). Our results suggested that eosinophils might promote angiogenesis[8].

Table IV. Blood-borne cells parameter in ischemic stoke patients. Values represent mean ± SD.

Variables	G1	G2	G3	G4	G5	G6
Eosinophils absolute value	0.2 ± 0.03	0.63 ± 0.11*	0.01 ± 0.06	0.3 ± 0.09	0.61 ± 0.12*	0.01 ± 0.08
Plateletcrit (PCT)	0.17 ± 0.13	0.18 ± 0.11	0.17 ± 0.06	0.17 ± 0.12	0.18 ± 0.04	0.16 ± 0.07
Platelet distribution width (PDW)	17.4 ± 0.12	15.6 ± 0.03*	18.6 ± 0.07	18.1 ± 0.09	15.8 ± 0.16*	19.7 ± 0.14
Mononuclear cells in absolute value	0.51 ± 0.03	0.49 ± 0.06	0.53 ± 0.06	0.49 ± 0.01	0.51 ± 0.08	0.48 ± 0.11

*p: compared to G1 and G4, *$p < 0.05$.

This study underscores the importance to investigate the further relationship between eosinophils and endothelial function.

Why did the counts of eosinophils decrease in stroke patients? We argue that it may be due to eosinophil apoptosis or migration into tissues during the stroke. This suggestion is supported by emerging evidence from both human and animal studies that suggest an active participation of eosinophils in several physiological and pathological processes such as inflammations and tissue remodeling[1]. It has been observed that the blood-brain barrier (BBB) is compromised during the ischemic stroke. Because of BBB breakdown, blood-borne immune cells infiltrate into the ischemic brain. Consequently, these infiltrating immune cells and damaged brain cells release inflammatory mediators that cause brain edema or directly promote the death of brain cells in the penumbra, thereby resulting in secondary progression of infarct lesions[9]. Sun et al[10] reported that eosinophils appeared in the post-ischemic cortex and was associated with neuronal death even in the absence of infarction, which in turn cause cortical atrophy. Why did stroke patients who had lower eosinophil counts, as compared to those with higher eosinophil counts, have much worse outcomes? It might be due to the release of cytokines and chemokines from eosinophils[1], such as IL-4[11-12], IL-6[13]and IL-10[14] which have been thought to have a damaging role in ischemic stroke. However, the reason for the change of eosinophil counts in stroke patients needs further investigation.

Platelet distribution width (PDW) is one of the indicators that reflect platelet activity[15-17]. Although previous studies demonstrated the platelet activation in patients with several kinds of diseases[18-21], a relationship between platelet factors in patients with stroke did not to have been specifically investigated. In our data, we investigated the association between PDW and outcome of stroke patients, evaluated with NIHSS scores (shown in Table IV). Our results show larger platelets are a risk factor for developing stroke, leading to worse outcome. We also analysed the covariates that predicted NIHSS (Table V) by A forward stepwise regression, which identified PDW is one of the predictors of NIHSS scores.

We observed alterations in neutrophil and monocyte counts. These changes were accompanied by variations in basophils, eosinophil, and platelet counts. However, the changes of neutrophil counts do not correlate with disease outcomes (Data not shown). Furthermore, although monocytes have shown to play a crucial role in post-ischemic inflammation[22-24], our data do not suggest that monocyte counts are prediction factors for outcomes in stroke patients (Table IV).

Conclusions

After analysis of related factors of NIHSS score in 3 months, our data indicate that eosinophil counts in stroke patients can predict the patients' outcome (Table V). In addition, the eosinophil counts are also associated with platelet activity. To our knowledge, we have for the first time shown eosinophil but not monocyte counts are related to organ malfunction. Out data, therefore, underscore the importance to investigate the further role of eosinophils in ischemic stroke.

Table V. Analysis of related factors of NIHSS score in 3 months.

Variables	Freedom	Regression coefficient	SEM	Wald chi-square value	*p*-value	Odds ratio value
Intercept	1	.652	.077	71.091	.000	
Age	1	.367	.038	93.257	.000	1.443
Sex	1	-.023	.019	1.426	.232	.977
Diabetes mellitus	1	.536	.052	106.774	.000	1.709
Hypertension	1	.535	.044	148.018	.000	1.708
infection	1	.012	.039	.097	.755	1.012
PCT<0.19%	1	.416	.049	108.133	.000	1.813
Eosinophils absolute value<0.02X10^9/L	1	.523	.036	99.701	.000	1.732
Eosinophils absolute value > 0.5X10^9/L	1	-.026	.022	1.372	.218	.986

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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