

# Platelet Aggregation and Interleukins Indicators Impacting the Outcomes of Ischemic Stroke

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*Aim:* Relative assessment and relationship between of platelet aggregation and the level of interleukins IL-1 $\beta$ , IL-4, IL-6, and IL-18 among ischemic stroke (IS) patients. *Methods:* A prospective clinical cohort study involved 108 IS patients, classified into group 1—surviving (93; 86.1%) and group 2—lethal outcomes (15; 13.9%). The studies were conducted in the most acute (first day of hospitalization) and acute (7 days of hospitalization) phases of IS. The level of interleukins was defined by enzyme immunoassay method. The platelet aggregation research was performed by the nephelometric method. *Results:* In the acute phase of the IS the first group, patients showed reduction in the level of IL-1 $\beta$  and IL-6, and increase in the level of IL-4, compared to the most acute period, whereas in patients of the second group further increase in IL-1 $\beta$  and IL-6, and reduction of IL-4 levels were recorded. Both in the most acute and acute phases of the IS, in parallel to the aggravation of platelet aggregation (PA) disturbances, the lethal outcome probability grew. The latter also grew together with increase in the synthesis of IL-1 $\beta$  and IL-6, in the most acute phase of the IS. In the acute phase of the IS, lethal outcome was recorded in all patients with high values of IL-1 $\beta$  and IL-6, and all patients with low values of IL-1 $\beta$  showed improvement. *Conclusions:* Vivid disturbances of PA, IL-1 $\beta$ , IL-6, and IL-18 synthesis, in the most acute phase of the IS, signal higher probability of lethal outcome. Reduction in the levels of IL-1 $\beta$ , IL-6, and IL-18, and increase of IL-4 in the acute phase, means improvement in conditions.

**Key Words:** Interleukins—ischemic stroke—neurological status—platelet aggregation

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## Introduction

The prevalence, mortality rate, and high probability of disability due to cerebrovascular diseases and their most severe manifestation, the stroke, prove that their prevention and treatment are the most urgent medico-social issues of today.

According to WHO bulletin, there were 6.24 million stroke deaths in 2015.<sup>1</sup> The map of stroke deaths in

countries broken down by income levels is worth considering. Respectively, 49.6 stroke deaths per 100,000 of population in low income countries for 2015; and ~1.5 times more in countries with lower-middle-income levels (e.g., Armenia), comprising 68.8 per 100,000 of population. In countries with medium-high income that indicator reaches 120.9 per 100,000 of population; and finally, in countries of high income it drops to 64.7 per 100,000 of population.<sup>2</sup>

Various studies have found that disorders in platelet aggregation (PA)<sup>3-7</sup> play a substantial role in the pathogenesis of ischemic stroke (IS). The fact that proinflammatory cytokines instigate and nourish the inflammatory reaction in the ischemic nidus and result in death of neurons, arduous clinical courses and inauspicious outcomes, has also been proven.<sup>3,8-15</sup>

A variety of cytokines triggers the inflammatory response to ischemic brain injury. The most important, proinflammatory cytokines are TNF- $\alpha$ , IL-1, and IL-6, which were detected within the brain, the cerebrospinal fluid, and the blood of patients suffering from acute IS

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and are responsible for the extension of the infarct zone in animal models.<sup>10</sup>

A nonspecific systemic inflammatory response occurs after both ischemic and hemorrhagic stroke, either as part of the process of brain damage or in response to complications such as deep venous thrombosis. Several studies reported that plasma levels of TNF- $\alpha$  and IL-6 are associated with prognosis after IS.<sup>11</sup> Among recent lacunar stroke patients, IL-6 and TNF receptor concentrations predict risk of recurrent vascular events, and they are associated with the effect of antiplatelet therapies.<sup>15</sup> Inflammation in IS is characterized by a storm of cytokines, chemokines, and damage-associated molecular patterns released by several cells contributing to exacerbate the tissue injury both in the acute and reparative phases.<sup>16</sup>

According to Fujimi et al. activated platelets release the transforming growth factor of fibroblasts TGF- $\beta$ 1 and other cytokines that regulate the body's immune response.<sup>17</sup> Chamorro<sup>18</sup> suggests that reactivation makes the platelets produce a number of proinflammatory cytokines.

Our previous studies showed that disorders in PA, in the most acute phase of IS, are accompanied by increased levels of proinflammatory interleukin IL-1b; the acute phase of IS, with reduced PA, is accompanied by lower level of IL-1b and increase in anti-inflammatory interleukin IL-4.<sup>19</sup> Respectively, the possible connection between PA indicators and interleukins to the outcomes of IS, is of interest.

Aim: relative assessment and relationship between of PA indicators and IL-1 $\beta$ , IL-4, IL-6, IL-18 interleukins levels among the surviving and nonsurvivors IS patients.

### Patients and Methods

The ongoing prospective clinical cohort study involves 108 IS patients, treated at the vascular neurology department of "St. Gregory the Illuminator" Medical Centre (Yerevan, Armenia), in the period of 2010-2013. This center is state-owned institutions providing medical management of the prevention, clinical intervention, and rehabilitation of patients with IS. All patients or relatives and patients' trustees provided signed informed consent. There is agreement of the Ethics Committee of Yerevan State University named after Mkhitar Heratsi.

*Inclusion criteria:* Hemispheric IS, hospitalization in the first 24 hours of stroke progress, surviving through the seventh day of hospitalization.

*Exclusion criteria:* Hospitalization after the first 24 hours; myocardial infarction; severe coronary artery disease; severe form of diabetes mellitus; hepatic failure; renal failure; malicious tumors; psychiatric diseases; infectious diseases; inflammatory diseases; pregnancy; serious heart, lung, kidney or other organ dysfunction; serious trauma or major surgery within the previous 3 months; platelet count <100.000/mm<sup>3</sup>.

All patients received anticoagulant and antiplatelet therapy in accordance with the clinical guidelines for the early management of patients with acute IS.<sup>20</sup> Thus, all studies were conducted against the background of therapy. However, the first blood sampling was performed before treatment.

### Patients

The surveyed cohort had 59 (54.6%) males and 49 (45.4%) females, in the age group of 30-90, the average being 67.56  $\pm$  11.34 years (95% confidence intervals [CI] from 65.38% to 69.56%). Table 1 contains data on the field and age of patients of the IS of our cohort.

Table 1 shows that the frequency of IS increases with the age of patients, reaching its peak (47.2%) at the age of 70-79 years. The youngest age was recorded in a man—30 years. In women, the earliest age was 51 years. In the age group 60-69 years, the incidence of IS in men is 3.5 times higher than in women (35.6% and 12.2%, respectively). In older age groups, the incidence among women begins to prevail. Thus, in the age group of 70-79 years, the incidence of IS in women is 2.4 times higher than in men (73.5% and 25.4%, respectively).

Ninety-three (86.1%) patients were discharged with improved health conditions, and lethal outcome was recorded among the remaining 15 patients (13.9%). Two groups of patients were formed based on the progress of the disease: survivors and nonsurvivors.

### Methods

Evaluation of the neurological status was performed using the National Institute of Health Stroke Scale (NIHSS) scale developed by the National Institute of Health (USA). The level of interleukins in blood serum was defined by enzyme immunoassay test with the use of Russian "Vector-Best" testing system. The results were expressed in pg/mL. The study of adenosine diphosphate (ADP)-induced PA was performed by the nephelometric

**Table 1.** Gender and age characteristics of patients with ischemic stroke

Gender	Age (years: n (%))					Total
	30-49	50-59	60-69	70-79	80-90	
Male	7 (11.9)	12 (20.3)	21 (35.6)	15 (25.4)	4 (6.8)	59 (100)
Female	0 (.0)	2 (4.1)	6 (12.2)	36 (73.5)	5 (10.2)	49 (100)
Total	7 (6.5)	14 (13.0)	27 (25.0)	51 (47.2)	9 (8.3)	108 (100)

method of Born,<sup>21</sup> with the help of “BIOLA” aggregation analyzer. The results were expressed in percentages. The study was conducted in 2 phases: the most acute phase (1 day of hospitalization) and acute phase (7-10 days after hospitalization). A sample of blood for analysis was taken in the morning before patients' medication intake.

#### Statistical Analyses

The statistical elaboration of study results was performed by IBM SPSS Statistics for Windows, Version 21.0 (SPSS, Inc., Chicago, IL). The verification of normal distribution of variables was done by one-sample  $\chi^2$  test and by Kolmogorov-Smirnov test. The comparison of PA and interleukins between the first and second groups of patients was conducted with the help of Mann-Whitney *U* test. The results are shown in frequency (%), medians (Me), interquartile range (IQR,  $Q_1$  and  $Q_3$ ), and 95% CI. The significance level for all statistical tests was set at  $P < .05$  (2-tailed).

## Results

#### Epidemiological Aspects of the Problem

In Armenia, from 2014 to 2017, there is a steady increase in the indices of both incidence and prevalence of IS. Thus, the incidence of IS, which in 2014 made 1026 cases, increased in 2017-1137 cases. Per 100,000 population, these indicators are 34.0 and 38.0, respectively. Prevalence of IS, which in 2014 was 2320 cases, also increased in 2017-2675 cases. Per 100,000 population, these indicators are 77.0 and 89.4, respectively. Mortality from IS, which in 2014 was 1287 cases, while, on the contrary, decreased in 2017-1201 cases. Per 100,000 population, these indicators are 42.7 and 40.1, respectively.

#### Risk Factors for IS and Confounding Factors

Identifying risk factors for IS is the basis for stroke prevention strategies. Modifiable risk factors for IS include hypertension, cardiac disease, atrial fibrillation, diabetes mellitus, hypercholesterolemia, frequent stresses, cigarette smoking, and alcohol abuse. The main modifiable risk factors for the development of IS in patients of our cohort were, to a greater extent, atherosclerosis, hypertension, ischemic heart disease, diabetes and their various combinations and, to a lesser extent, smoking and stress, which were risk factors, mainly in young men.

We tested the relationship between risk factors for IS and the likelihood of a lethal/favorable outcome.

Table 2 shows that the relative risk of the favorable outcome is 1.176 with 95% CI from 1.083 to 1.277. This means that risk factors for IS affect the likelihood of an outcome. Nevertheless, the CI range includes 1, and hence, the result is statistically insignificant. Despite this, the width of CI in 95% of cases allows to declare the results clinically important.

**Table 2.** Relative risk of the lethal/favorable outcome, depending on the risk factors for ischemic stroke

Risk Estimate	Value	95% CI	
		Lower	Upper
For cohort outcome = favorable outcome	1.176	1.083	1.277

In addition to the risk factors, research objects can be characterized by a number of other unaccounted factors (the so-called “confounding factor,” or “confounder”), whose influence may distort the effect of the risk factors. Such factors can lead to the appearance of a systematic error related to confounding bias.

We analyzed the frequency of occurrence of confounders in our cohort of IS patients. The analysis showed that 80 (74.1%) patients had some confounders. In 28 (25.9%) patients, concomitant pathology was not observed. However, this is provided that we do not take into account atherosclerosis, hypertension, diabetes mellitus, and coronary heart disease as confounders. If these pathologies were taken into consideration, then the confounders were present in the 101st (93.5%) IS patients.

#### Clinical and Ethnic Characteristics

Initially this study included 132 IS patients, treated at the vascular neurology department of “St. Gregory the Illuminator” Medical Center, in the period of 2010-2013. Ninety-seven IS patients (73.5%) had a favorable outcome of the disease, and 35 (26.5%) had a lethal outcome. Patients died at various times after hospitalization, and therefore these cases were excluded from the study. Another 4 IS patients drop out from the study due to refusal of treatment. Thus, 108 IS patients were examined at both phases of the study.

According to the ethnicity of 108 IS patients distributed as follows: 104 (96.3%)—Armenians; 2 (1.9%)—Yezidis; 2 (1.9%)—Russians. Thus, it can be said that all results of the analysis were obtained for IS patients from the Armenian population living in the territory of Armenia.

#### NIHSS Indicators in Survivors and Nonsurvivors Patients in Most Acute and Acute Phases of IS

Table 3 contains descriptive statistics of NIHSS indicators, in patients of groups 1 and 2.

Table 3 shows that the median NIHSS<sup>1</sup> score of surviving IS patients in the most acute phase was 11 (IQR, 10-12), the median NIHSS<sup>2</sup> score of surviving IS patients in the acute phase was 6 (IQR, 5-7), whereas the median NIHSS<sup>1</sup> score of nonsurviving IS patients in the most acute phase was 13 (IQR, 12-14), the median NIHSS<sup>2</sup> score of surviving IS patients in the acute phase was 16 (IQR, 14-17).

**Table 3.** NISS indicators in patients of groups 1 and 2, in the most acute and acute phases of ischemic stroke

Indicators	n	Me	Q <sub>1</sub>	Q <sub>3</sub>	
Group 1	NIHSS <sup>1</sup>	93	11	10	12
	NIHSS <sup>2</sup>	93	6	5	7
Group 2	NIHSS <sup>1</sup>	15	13	12	14
	NIHSS <sup>2</sup>	15	16	14	17

Note: figures in superscript are as follows: 1—most acute phase, 2—acute phase.

*Platelet Aggregation and Interleukins Indicators in Survivors and Nonsurvivors Patients in Most Acute and Acute Phases of IS*

Tables 4 and 5 contain descriptive statistics of PA and interleukins IL-1β, IL-4, IL-6, IL-18 indicators, in patients of groups 1 and 2.

*Comparison of Platelet Aggregation and Interleukins Indicators in Survivors and Nonsurvivors Patients in the Most Acute and Acute Phases of IS*

Tables 6 and 7 shows the comparison of PA and interleukins IL-1β, IL-4, IL-6, IL-18 indicators, according to Mann-Whitney U test.

Table 6 shows that PA indicators of the second group in the most acute phase are higher than those of the first group (U = 957.000, Z = 2.307, P = .021). Indicators IL-1b and IL-6 in patients of the second group are higher than those of the first group patients (U = 1282.00, Z = 5.198, P < .001; U = 1145.00, Z = 3.976, P < .001, respectively). There is no statistically significant relationship between IL-4 and IL-18 (U = 612.500, Z = -.765, P = .444; U = 786.00, Z = 1.262, P = .207, respectively).

Table 7 shows that in the acute phase the PA indicators in second group patients are noticeably higher than those of the first group patients (U = 1375.000, Z = 6.023, P < .001). IL-1b, IL-6, and IL-18 indicators in second group patients are higher than those of the first group

patients (U = 1395.00, Z = 6.214, P < .001; U = 1374.00, Z = 6.010, P < .001; U = 1052.50, Z = 3.780, P < .001, respectively). The IL-4 in second group patients, on the contrary, was significantly lower in the acute phase than in the first group patients (U = 13.500, Z = -6.081, P < .001).

**Discussion**

The indicators of PA in IS patients of the second group in the most acute phase were higher than those of the first group. IL-1b and IL-6 indicators in IS patients of the second group were noticeably higher than those of the first group. Further growth of PA, IL-1b, and IL-6 indicators in the acute phase was observed in IS patients of the second group, unlike those in the first group.

The opposite situation occurred when analyzing IL-4 indicators. In the most acute phase of IS, these indicators did not differ from one another, in both groups. However, in the acute phase IL-4 started to drop in the patients of the second group, unlike those of the first group. IL-18 indicators in 1 and 2 group patients in the most acute period of IS were practically the same.

Hence, the results of our survey prove the opinion that PA grows in the most acute phase of the IS, as well as the synthesis of proinflammatory interleukins IL-1b and IL-6. One may conclude that between these 2 processes: platelets activation, aggregation, degranulation, and interleukin synthesis, there is a mutual relation, namely lysis of aggregated platelets is accompanied by the expression of the synthesis of proinflammatory interleukins.

Essential differences in the indicators of PA, IL-1b, and IL-6 in both groups of patients were also identified, specifically in the most acute phase of the IS these indicators were significantly higher among the exiting patients, compared to those surviving.

In the most acute phase of the IS, there is statistically relevant connection between the indicators of IL-1β, IL-6 interleukins, and the outcome, exactly the higher is the

**Table 4.** Platelet aggregation and interleukins indicators in patients of group 1, in the most acute and acute phases of ischemic stroke

Indicators	n	Me	Q <sub>1</sub>	Q <sub>3</sub>	95% CI (Me)	
					Lower	Upper
PA <sup>1</sup> (%)	93	27.30	26.35	28.30	26.80	27.80
PA <sup>2</sup> (%)	93	11.90	11.40	12.40	11.70	12.10
IL-1b <sup>1</sup> (pg/mL)	93	14.50	11.20	19.90	12.00	18.00
IL-4 <sup>1</sup> (pg/mL)	93	1.10	1.08	1.20	1.09	1.10
IL-6 <sup>1</sup> (pg/mL)	93	18.90	14.70	26.40	17.40	20.80
IL-18 <sup>1</sup> (pg/mL)	87	120.50	38.90	267.90	65.70	182.10
IL-1b <sup>2</sup> (pg/mL)	93	10.00	9.00	11.00	9.20	10.20
IL-4 <sup>2</sup> (pg/mL)	93	2.16	2.07	3.08	2.09	3.01
IL-6 <sup>2</sup> (pg/mL)	93	10.50	7.05	17.30	9.90	12.30
IL-18 <sup>2</sup> (pg/mL)	87	90.20	23.00	252.50	55.50	134.40

**Table 5.** Platelet aggregation and interleukins indicators in patients of group 2, in the most acute and acute phases of ischemic stroke

Indicators	n	Me	Q <sub>1</sub>	Q <sub>3</sub>	95% CI (Me)	
					Lower	Upper
PA <sup>1</sup> (%)	15	28.20	27.40	28.60	27.41	28.60
PA <sup>2</sup> (%)	15	36.40	25.00	39.80	25.00	38.90
IL-1b <sup>1</sup> (pg/mL)	15	28.20	23.80	34.20	23.80	31.50
IL-4 <sup>1</sup> (pg/mL)	15	1.09	1.07	1.10	1.07	1.10
IL-6 <sup>1</sup> (pg/mL)	15	65.80	23.30	102.00	32.50	102.00
IL-18 <sup>1</sup> (pg/mL)	14	146.00	130.80	253.20	130.80	253.20
IL-1b <sup>2</sup> (pg/mL)	15	28.40	24.80	34.80	26.30	32.20
IL-4 <sup>2</sup> (pg/mL)	15	1.02	1.00	1.06	1.00	1.02
IL-6 <sup>2</sup> (pg/mL)	15	105.80	67.70	112.80	95.40	112.40
IL-18 <sup>2</sup> (pg/mL)	10	265.00	176.70	365.70	176.70	364.94

**Table 6.** Comparison of platelet aggregation and interleukins indicators in patients of groups 1 and 2, in the most acute phase of ischemic stroke

Null hypothesis	Mann-Whitney U-test	Standardized statistics	P value	Decision
The distribution of PA is the same across categories of first and second groups	957.000	2.307	.021	Reject the null hypothesis
The distribution of IL-1b is the same across categories of first and second groups	1282.000	5.198	<.001	Reject the null hypothesis
The distribution of IL-4 is the same across categories of first and second groups	612.500	-.765	.444	Retain the null hypothesis
The distribution of IL-6 is the same across categories of first and second groups	1145.000	3.976	<.001	Reject the null hypothesis
The distribution of IL-18 is the same across categories of first and second groups	786.000	1.262	.207	Retain the null hypothesis

**Table 7.** Comparison of platelet aggregation and interleukins indicators in patients of groups 1 and 2, in the acute phase of ischemic stroke

Null hypothesis	Mann-Whitney U test	Standardized statistics	P value	Decision
The distribution of PA is the same across categories of first and second groups	1375.000	6.023	<.001	Reject the null hypothesis
The distribution of IL-1b is the same across categories of first and second groups	1395.000	6.214	<.001	Reject the null hypothesis
The distribution of IL-4 is the same across categories of first and second groups	13.500	-6.081	<.001	Reject the null hypothesis
The distribution of IL-6 is the same across categories of first and second groups	1374.000	6.010	<.001	Reject the null hypothesis
The distribution of IL-18 is the same across categories of first and second groups	1052.500	3.780	<.001	Retain the null hypothesis

synthesis of these interleukins, the higher is the probability of lethal outcome. There is no statistically relevant connection between the IL-4, IL-18 indicators, and the IS outcome.

The results of our study also showed the unstable character of interleukins dynamics in the acute phase of the IS, compared to the most acute phase. The surviving patients demonstrated reduction in the level of proinflammatory interleukins IL-1b, IL-6, and IL-18, and on the opposite,

increase in the level of anti-inflammatory interleukin IL-4. In the same time, the exiting patients demonstrate further increase in the levels of IL-1b, IL-6, and IL-18, and reduction in IL-4.

**Conclusions**

We may conclude that reduction in platelet aggregation and proinflammatory interleukins IL-1b, IL-6, and IL-18

in surviving IS patients in the acute phase, and increase in the level of anti-inflammatory interleukin IL-4 are accompanied with improvements in clinical condition. In exiting patients the negative dynamic of these indicators occurs along the aggravation of the clinical condition. In the acute phase of the IS, decrease in the levels of IL-1 $\beta$ , IL-6, IL-18 contributes to a higher probability of a favorable outcome. Increase of the IL-4 in the acute phase of the IS, in its turn, also proves the high probability of a favorable outcome.

### Consent

All patients gave their written informed consent to participate in this study. Ethics Committee of Yerevan State University has approved the implementation of this research.

### Conflict of Interest

All authors claim that there are no conflicts of interest.

### Authors' Contributions

Rouben Hovhannesian combined the bibliography, developed the study concept and design, analyzed and interpreted the data, wrote the paper, and designed the tables. Iren Hovhannesian did the research and interpreted the data.

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