The Relationship Between Immune and Inflammatory Markers and Short-term Clinical Outcomes after Stroke: Side Matters

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ABSTRACT **Background:** The two cerebral hemispheres influence the immune response differently. While the left hemisphere enhances cellular immunity, the right hemisphere inhibits it.

Objectives: To determine whether immune and inflammatory markers correlated with stroke severity and hospitalization duration as a function of stroke side.

Methods: The study included 137 patients with unilateral ischemic stroke. The medical records were reviewed for demographic and clinical laboratory data, including C-reactive protein (CRP), white blood cell (WBC) count, its differential stroke side and stroke severity according to the National Institute of Health Stroke Scale (NIHSS), and length of hospital stay (LOS). We examined differences between right side (RS) and left side (LS) stroke on immune and inflammatory markers and compared correlations between these markers and NIHSS and LOS as a function of stroke side.

Results: RS stroke patients had higher CRP and monocytes than LS stroke patients. In RS stroke patients, CRP, total WBC, and lymphocyte levels positively correlated with both NIHSS and LOS, whereas levels of neutrophils were positively correlated with NIHSS alone. No correlations were found for LS stroke patients.

Conclusions: Immune-inflammatory markers correlated with stroke severity and LOS only in patients with RS stroke. Neuroimmunological processes influence short-term clinical outcomes after stroke, especially considering the differential effects of the hemispheres on immunity. Prospective studies that evaluate long-term clinical outcomes are needed. Testing the effects of anti-inflammatory treatments on prognosis of RS stroke patients should be considered.

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The nervous and immune systems constantly influence each other bi-directionally. The nervous system innervates lymphoid glands, and immune cells express receptors for neurotransmitters [1]. The immune system communicates with the brain via direct penetration of the brain in regions lacking the blood brain barrier (BBB), via monocyte *crosstalk* on both sides of the BBB, and via the vagal nerve [2]. The vagal nerve transmits information about peripheral inflammation via receptors for interleukin-1 and then regulates peripheral inflammation by the hypothalamic-pituitary-adrenal (HPA) axis secretion of cortisol and by the descending vagus nerve route. The latter signal reaches the spleen via a conversion of vagal to sympathetic signaling. Using specific splenic T-cells, this pathway inhibits inflammation synthesized by macrophages [3]. However, another and higher-order neural regulatory mechanism of immunity involves the two hemispheres.

Hemispheric lateralization (HL) refers to the tendency to activate parts or use functions associated more with one hemisphere than the other. While the left hemisphere increases cellular immunity, the right hemisphere inhibits it. This finding was supported in animals and in a systematic review study in humans, which found that left-HL was related to higher cellular immunity while right-HL had the opposite effect [4]. One study found that levels of left HL, as measured by resting electroencephalogram, were positively correlated with levels of natural killer cells [5]. Another study, which examined this in epileptic patients undergoing therapeutic protective surgical lesions, demonstrated that left hemisphere lesions led to reduced lymphocyte and helper T-cell levels while right hemisphere lesions led to increases in those immune parameters [6]. Lesions presumably induce reduced cerebral activity, and hence, these effects are in the opposite direction than the differential effects of normal hemispheric activity on immunity [4].

Concerning the relationships between stroke laterality and immunity, an early study examined differences in T-cell mediated delayed-type hypersensitivity (DTH) between right side

Table 1. Background and clinical characteristics of patients as function of stroke lateralization

Variable	Left hemisphere (n=75)	Right hemisphere (n=62)*	*P*-value
NIHSS categories, n (%)			
1	0 (0)	0 (0)	0.63
2	44 (61.10)	31 (54.40)	
3	24 (33.30)	24 (42.10)	
4	3 (4.20)	2 (3.50)	
5	1 (1.40)	0 (0)	
Sex (%)			
Male	66.7	48.1	0.03
Female	33.3	51.9	
Ethnicity (%)			
Jewish	49.33	68.52	0.02
Arab	50.67	31.48	
Co-morbidities (%)			
Hypertension	68.0	85.2	0.03
Psychiatric disorders	1.3	1.9	0.81
Atrial fibrillation	10.7	24.1	0.04
Diabetes	42.7	50	0.41
Cardiovascular disease	10.7	14.8	0.48
Ischemic heart disease	17.3	27.8	0.16
Vascular disease	6.7	5.6	0.80
Smoking	26.7	7.4	0.00
Alcohol abuse	0	1.9	0.24
Obesity	17.3	7.4	0.10
Hyperlipidemia	60	68.5	0.32

*For some variables, the sample slightly changed due to missing data

NIHSS = National Institutes of Health Stroke Scale

(RS) and left side (LS) strokes. DTH of antigen-specific T-cell responses in the affected paralyzed right limb (after LS stroke) were stronger than in the left paralyzed side (after RS stroke) [7]. In contrast, another study found that RS stroke patients had higher left paretic side DTH [8]. This finding was supported by Minnerup et al. [9] who found that LS stroke patients exhibited more pneumonia, possibly pointing to initial immune suppression. However, this relationship did not remain statistically significant in a multivariate analysis controlling for confounders [9]. These three studies are contradictory, but the last two suggest that stroke lesions impair a hemisphere and are thus in line with left hemisphere immune potentiation when that hemisphere is intact [4].

Right-handed ischemic stroke patients have a stronger correlation between C-reactive protein (CRP) and white blood cell (WBC) counts as well as a higher degree of variability of CRP and WBC than RS stroke patients [10]. Elevated levels of inflammatory markers have been found in stroke patients including CRP, interleukin (IL)-6 and IL-12, and other anti-inflammatory cytokines (IL-1Ra, IL-10). However, these markers were not associated with stroke lateralization. Such inconsistency in our study results may indicate possible associations between immune and inflammatory parameters separately in LS and RS stroke patients.

We found only one study [10] that examined whether stroke lateralization moderates or changes any associations between immune and clinical parameters relevant to stroke. Studies on stress and mental health have shown that more severe life events correlated with poor mental health, but only in right-HL patients [11]. Since the inflammatory response plays an etiological role in stroke and given the moderating role of HL in relationships between threat and mental health [11], it is possible that the relationship between immune and inflammatory markers with disease severity differs in RS vs. LS strokes. Thus, we examined the levels of immune and inflammatory markers in LS vs. RS stroke patients and the correlations between these immune parameters and short-term clinical outcomes following RS vs. LS strokes, separately.

PATIENTS AND METHODS

METHODS AND STUDY DESIGN

Sample

This retrospective study included 137 patients with unilateral ischemic stroke admitted to the Ziv Medical Center, Israel, between May 2014 and December 2015. Patients were selected according to the following inclusion criteria diagnosis of a unilateral ischemic stroke verified by computed tomography (CT) scans, CT-angiogram, and magnetic resonance imaging (MRI) scans, as well as clinical diagnosis by a neurologist. Exclusion criteria included hemorrhagic or uncertain etiology of stroke, bilateral stroke, transient ischemic accident, and an additional major neurological co-morbidity (e.g., brain injury, epilepsy). This sample size follows the recommended 15–20 participants per variable since we had four predictor variables and two outcomes. The study was approved by the internal ethics committee board at the Ziv Medical Center.

Design

In a combined cross-sectional and brief longitudinal correlational design study, we examined the associations between various

variables at time one (e.g., WBC count with disease severity) as well as the prospective association between immune variables obtained at time 1 and length of hospital stay (LOS) assessed at time 2. This study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies. Predictor variables included the immune and inflammatory variables, while the outcome variables were the National Institutes of Health Stroke Scale (NIHSS) disease severity score and the LOS.

Measures

Background data included the included patient's age, sex, smoking history, alcohol consumption, stroke side and localization (anterior, posterior), and co-morbidities. Immune and inflammatory measures included immune parameters such as total WBC count and its differential including absolute and percentages of monocytes, lymphocytes, and neutrophils. The inflammatory index was CRP. Clinical outcomes included days in hospital and severity of stroke as assessed by the National Institute of Health Stroke Scale (NIHSS). Higher scores indicate a poorer clinical status. The original stroke severity score (NIHSS) is a continuous variable between 0 and 42. We followed the common clinical practice, which classifies patients into five categories according to the NIHSS score: 1 = a score of 0 with no stroke symptoms; 2 = a score of 1–4, minor stroke; 3 = a score of 5–15, moderate stroke; 4 = a score of 16–20, moderate-severe stroke; and 5 = a score of 21–42, severe stroke. Although the new score is ordinal, since it is based on an initial interval scale (0–42), we treated this variable as a continuous one, performing Pearson correlation tests rather than chi-square tests. The results using the more continuous score of 0–42 yielded equivalent results.

STATISTICAL ANALYSES

Continuous variables were described using means and standard deviations, while categorical variables were expressed as percentages of cases. To examine differences on all collected data between RS and LS strokes, *t*-tests were used for continuous variables and chi-square tests for categorical variables. Pearson correlation tests showed associations between immunological/inflammatory variables and the clinical outcomes (stroke severity and hospitalization days), separately for left and right-sided strokes.

RESULTS

We initially recruited 151 patients, of whom 137 patients who had CRP data; 75 (54.7%) had LS strokes, while 62 (45.3%) had RS strokes. The mean age for the entire sample was 70.71 ± 11.83 years.

STROKE SIDE AND BACKGROUND VARIABLES

For the background variables, no statistically significant differences were found between RS and LS stroke patients on several background variables (all *P*-values > 0.05), except for the following variables: Patients with RS strokes had a significantly higher prevalence of atrial fibrillation and hypertension, smoked less frequently, and were more likely to be Jewish and female compared to patients with LS strokes. In addition, age significantly differed between stroke side groups: 85.2% of RS stroke patients were 65 years or older. Last, no differences were found between stroke side groups on stroke severity (chi-square 1.73, $P > 0.05$) [Table 2].

STROKE SIDE, IMMUNE AND INFLAMMATORY MARKERS, AND CLINICAL OUTCOMES

Concerning differences in immune and inflammatory parameters, RS stroke patients had significantly higher levels of CRP (t[75.84] = 2.01, $P < 0.05$) and monocytes (t[64.68] = 2.34, $P < 0.05$) than LS stroke patients [Table 2].

Last, we examined the correlations between these immune and inflammatory parameters and stroke severity (NIHSS) as well as LOS as outcome measures separately for each stroke side group. The study results revealed a positive and significant correlation between CRP levels, total WBC, lymphocytes, and two outcome measures, namely NIHSS and LOS, but only in patients with RS stroke. Levels of neutrophils were positively and significantly correlated only with stroke severity (NIHSS) [Table 3]. In contrast, no correlations between the immune/inflammatory markers and the two clinical outcomes were observed in patients with LS stroke.

Table 2. Differences between patients with left vs. right hemisphere strokes on continuous background, immune, and clinical variables

	Left hemisphere (n=75)	Right hemisphere (n=62)
Variable	Mean ± SD	Mean ± SD
Age, in years	67.8±11.99	74.74 ± 10.42
NIHSS (1–5)	2.46 ± 0.65	2.49 ± 0.57
C-reactive protein	8.52 ± 12.33	17.19 ± 32.12*
Total WBC	8.30 ± 2.35	8.76 ± 3.81
Neutrophils	5.58 ± 1.96	5.60 ± 2.30
Lymphocytes	2.00 ± 1.03	2.37 ± 2.50
Monocytes	0.45 ± 0.16	0.68 ± 0.74*
Hospitalization days	6.29 ± 3.42	6.72 ± 4.08

NIHSS = National Institutes of Health Stroke Scale, SD = standard deviation, WBC = white blood cells count

*$P < 0.05$

DISCUSSION

In this study, we demonstrated that RS stroke patients had higher levels of peripheral inflammation, based on CRP and monocyte levels, compared to LS stroke patients. Furthermore, only in RS stroke patients did positive correlations emerge between inflammatory/immune parameters and severity of stroke. Specifically, only in RS stroke patients there were significant and positive correlations between CRP, total WBC, and lymphocytes, with indexes of clinical outcomes, namely stroke severity (NIHSS) and LOS. Neutrophil levels correlated significantly and positively with NIHSS level in RS stroke patients as well. In contrast, no such associations between immune/inflammatory indexes and the short-term clinical outcomes were found for patients with LS stroke. RS stroke patients were older and had a higher prevalence of atrial fibrillation and hypertension, but they smoked less than those with LS stroke.

How can this pattern of results be understood? The left hemisphere is known to increase peripheral cellular immunity including natural killer cells and T-cells [5,6]. A systematic review confirmed these results in 11 studies [4], showing cellular immune potentiation by the left hemisphere and immune suppression by the right hemisphere. Concerning inflammation, one study found that following a pathogenic challenge (lipopolysaccharide), levels of mRNA interleukin-6 (IL-6) were higher in mice astrocytes from the left compared to the right hemisphere [12]. Another stroke study found a biphasic inflammatory response. Neutrophils were increased immediately following the stroke and a week after the stroke they decreased below baseline levels without evidence of infections [13]. Importantly, the inflammatory response plays a crucial role in the pathogenesis and prognosis of strokes [14]. In contrast to Zhang [12], Shields and Moons [15] found that RS cerebral activity correlated with higher levels of IL-6 in healthy people without an immunological challenge. The conflicting evidence may result from methodological differences between these studies as some studies used animals while others tested human participants, and some used immune challenges while others examined natural levels of immune parameters without an immune challenge.

Since it is plausible that a stroke impairs affected hemisphere activity, it is possible that a RS stroke reduces the intact right hemisphere's immunosuppressive effects. This result could then explain our observation of higher inflammation among RS stroke patients. These results are also in line with Tarkowski and colleagues [7,8] who found greater T-cell mediated DTH in RS than in LS stroke patients.

Our results may also be explained by another mechanism. It is also possible that the autonomic nervous system, whose activity was not measured in the present study, may partly explain the results observed here. Several studies have demonstrated that the sympathetic nervous system is predominantly controlled by the brain's RS (e.g., the insular cortex), while the LS predominantly controls the parasympathetic nervous system [16]. The cerebral cortex in rodents shows lateral specialization in its regulation of immunity with immunosuppression being controlled by the right hemisphere, and immunopotentiation by the left one [17,18]. Humans show similar lateral specialized control of the immune system as seen in strokes [10], surgery to control epilepsy [6], and application of transcranial magnetic stimulation (TMS) [19]. A systematic review of human studies also supports this conclusion [4].

In addition, psychological stress is associated with higher pro-inflammatory and lower anti-inflammatory cytokines, especially in stress-prone individuals [20]. Last, the vagal nerve, which normally opposes the sympathetic response, has profound anti-inflammatory effects [3,14]. One study found that RS stroke induced decreases in parasympathetic vagal activity, indexed by heart rate variability (HRV) [7]. This pattern in return could show less vagal control of inflammation and thus to higher early inflammation, especially innate immunity manifested specifically by the observed elevated levels of monocytes in patients with RS stroke in the present study. We did not measure HRV in the present study. However, it is possible that RS stroke induced reduction in vagal neuroimmunomodulation. This possible reduction in vagal inhibition of inflammation, together with the observed higher monocytes, may have led to the positive correlations between inflammation and stroke severity seen only in RS stroke patients. Future studies need to verify this possible mechanism by measuring the vagal nerve index of HRV, stroke side, and more specific inflammatory markers.

With regard to the differences between lateralization sides and

Table 3. Pearson correlations between immune parameters and clinical outcomes in stroke patients according to their stroke side

Immune parameter	Stroke side	NIHSS	LOS
C-reactive protein§	Left	0.07	-0.04
	Right	0.36**	0.46***
White blood cells count§	Left	0.1	-0.04
	Right	0.30*	0.26*
Neutrophils	Left	0.10	0.10
	Right	0.36**	0.19
Lymphocytes	Left	0.07	-0.18
	Right	0.28*	0.39**
Monocytes	Left	-0.14	-0.22
	Right	0.02	-0.03

§log transformed due to non-normal distribution

*P < 0.05, **P < 0.01, ***P < 0.001

LOS = length of stay in hospital, NIHSS = National Institutes of Health Stroke Severity

background variables, RS stroke patients were older and more often presented with hypertension and atrial fibrillation, yet they less often smoked than those with LS stroke. There were also sex and ethnicity differences. These background factors were present prior to the stroke and may have partly acted as risk factors for RS stroke. These differences between stroke sides may possibly be explained as following: hypertension and atrial fibrillation are both associated with excessive sympathetic activity [21]. With regard to smoking, decreased RS activity as well as elevated LS activity were related to risk-taking behaviors and impulsivity, which may also be related to smoking [22]. Concerning the observed age differences, sympathetically related disorders were more frequent among the RS stroke patients who were also older, which corresponds to reductions in parasympathetic vagal tone with age [23]. Although vagal tone was not measured in the present study, it is possible that age-related reduction in vagal activity may also explain the greater inflammation observed in the present study among RS stroke patients who were also older [24].

The current study reflects an initial examination between stroke side, inflammation, and stroke severity. As an initial examination, the present study had a few limitations. First, we did not include measures of sympathetic and parasympathetic activity, which may account for the observed results. Second, we did not measure more specific immunological (e.g., T-cell subtypes) and inflammatory markers (e.g., IL-1, IL-6, IL-10), which may have revealed a more precise and possibly complex pattern of hemispheric differences and associations with the clinical outcomes. Third, our clinical outcomes were short-term. Future studies should measure long-term outcomes relevant to stroke such as CT or MRI-based measures of stroke size and location, modified Rankin scale (for measuring the degree of disability/dependence after a stroke), and survival. Last, we conducted a cross-sectional study. Future studies should examine prospectively whether early inflammatory parameters predict long-term clinical outcomes differently as a function of the stroke side.

CONCLUSIONS

RS stroke patients may have more inflammation than those with LS stroke. Inflammatory markers correlated with stroke severity and length of stay only in RS but not in LS stroke patients. These results reveal the relevance of neuroimmunology to understanding and possibly treating strokes. If these results are replicated in future prospective studies with long-term clinical outcomes, they could have significant therapeutic implications, highlighting the need for investigating the potential effects of anti-inflammatory treatments, such as specific medications or non-invasive vagal nerve stimulation, on the prognosis of patients with RS stroke.

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