Cortisol is More Important than Metanephrines in Driving Changes in Leukocyte Counts after Stroke

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Background: There are notable changes in the number of white blood cells (WBCs) after stroke, but the primary mediators of these changes are unclear. In this study, we assessed the role of the neuroendocrine and sympathetic nervous systems in stroke-induced changes of WBCs within distinct leukocyte subsets, as well as the effect of these changes on stroke outcomes. *Methods:* Patients were recruited within 72 hours after ischemic stroke; complete blood count with differential was obtained at set time points. The relationships among leukocyte numbers, cortisol, adrenocorticotropic hormone, interleukin-6, and metanephrines were assessed at 72 hours after stroke. Associations between abnormal leukocyte counts at 72 hours, poststroke infection, and 3-month outcomes were determined. *Results:* A total of 114 subjects were enrolled. Severe stroke was associated with leukocytosis, neutrophilia, monocytosis, lymphopenia, and eosinopenia. At 72 hours after stroke, increased serum cortisol was independently associated with neutrophilia and lymphopenia. Abnormal leukocyte counts were not independently predictive of poststroke infection, but lymphopenia was associated with poor outcome (modified Rankin score >3) at 3 months after stroke (odds ratio = 22.86 [1.95, 267.65]; $P = .01$). *Conclusions:* Increased serum cortisol is independently associated with neutrophilia and lymphopenia after stroke. Lymphopenia is not an independent predictor of infections but is independently associated with worse outcome. **Key Words:** Leukocytes—lymphocytes—eosinophils—cortisol—metanephrines.

A variety of immunologic perturbations occur after stroke, with one of the most notable being that of changes in the number of white blood cells (WBCs) within different leukocyte subsets. Data consistently show that stroke induces leukocytosis, which is predominantly driven by an increase in polymorphonuclear cells (PMNs) and, to a lesser extent, an increase in mononuclear cells.[1,2] At the same time, there is a decrease in the number of lymphocytes, and the degree of this decrease correlates with stroke severity.[1,3-6] The decrease in circulating lymphocytes is thought to be mediated by activation of the sympathetic nervous system as well as by an increase in systemic glucocorticoids.[7] Adrenocorticotropic hormone (ACTH) is produced and secreted by the anterior pituitary gland and is responsible for increasing the production and release of cortisol from the adrenal cortex. Following stroke, however, the elevation in cortisol appears to be partly mediated by interleukin-6 (IL-6).[8]

A standard leukocyte differential also includes information about the number of eosinophils. There are limited data regarding the contribution of eosinophils to stroke-related outcomes. Recent studies suggest that lower eosinophil numbers after stroke are associated with an increased risk of infection as well as worse outcomes.[9,10]

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These studies, however, did not control for stroke severity and have other methodological issues that limit interpretation of the findings.

In this study, we assessed the correlation among stroke severity, cell counts within distinct leukocyte subsets, the risk of infection, and functional outcome in patients with ischemic stroke. Further, we tested the strength of the association between these cell counts and systemic levels of cortisol, ACTH, IL-6, and metanephrines.

Methods

The patient cohort has been described elsewhere.[5,11] Briefly, patients with ischemic stroke admitted within 72 hours of symptom onset were enrolled in a prospective study evaluating postischemic immune responses. Patients with immunodeficiency (HIV) or on immunomodulatory treatments were excluded. The study was approved by the institutional review board; all patients or their surrogates provided informed consent.

Clinical Data

Stroke severity was determined by the National Institutes of Health Stroke Scale (NIHSS) score, and outcome was determined by the modified Rankin scale (mRS) at 3 months. Intervention was defined as the use of intravenous alteplase or endovascular therapy. Poor outcome was considered to be an mRS greater than 3. Infarct volume was calculated on diffusion-weighted magnetic resonance imaging by a neuroradiologist using the ABC/2 method.[12]

Laboratory Studies

WBC count and differential were determined by the clinical laboratory. Classification of abnormal cell counts was based on the laboratory's normative data as follows: WBCs greater than 10,000/μL, PMNs greater than 7000/μL, lymphocytes less than 1,000/μL, monocytes greater than 800/μL, and eosinophils less than 50/μL (there is no universally accepted lower limit for eosinophils, this number is thus somewhat arbitrary). The concentrations of ACTH and cortisol were also determined by the clinical laboratory using standard methodologies. IL-6 was measured with a cytometric bead-based system (Fluorokine MAP; R&D Systems, Minneapolis, MN); the lower limit of detection was 1.1 pg/mL. Standard enzyme-linked immunoassay was used to determine the plasma concentrations of plasma metanephrines (IBL-America, Minneapolis, MN; sensitivity = 14.9 pg/mL).

Statistics

Descriptive data are presented as mean ± standard deviation or median and interquartile range; group comparisons were performed using analysis of variance or the Kruskal-Wallis H test as appropriate. Correlations are presented using Spearman rho. Categorical data are compared by chi-square test. Logistic regression was used to assess the predictors of clinically abnormal leukocyte counts, infection, and poor outcome at 3 months after stroke onset. Biologically plausible variables were included in the models. Significance was set at $P < .05$.

Results

A total of 114 patients were enrolled. There was a prolonged elevation in plasma cortisol in patients with severe stroke (Fig 1, A). More severe strokes were also associated with an increase in WBCs (Fig 1, B), PMNs Fig 1, C), and monocytes Fig 1, D) that last for at least a week after stroke onset, as well as a more short-lived decrease in the number of lymphocytes and eosinophils (Fig 1, E,F). The correlations among ACTH, IL-6, cortisol, metanephrines, WBCs, and leukocyte subsets with stroke severity (NIHSS score, infarct volume) at 72 hours after stroke are shown in Figure 2. (The median value and interquartile range are provided for each biomarker of stress or inflammation.) ACTH and IL-6 are both highly correlated with cortisol levels, which in turn are highly correlated with stroke severity. In general, changes in PMNs and monocytes track together, whereas changes in lymphocytes and eosinophils track together, and all are highly correlated with both cortisol and IL-6, but not ACTH. Metanephrines were inversely correlated with lymphocyte and eosinophil numbers. "Clinically abnormal" cell counts are prevalent on the first day after stroke, whereas at least 50% of patients with severe stroke continue to manifest abnormal neutrophil and monocyte levels at day 3 after stroke (Table 1); laboratory-defined lymphopenia is less common than laboratory-defined neutrophilia and monocytosis. Table 2 shows the predictors of "clinically abnormal" cell counts at 3 days after stroke. After controlling for stroke severity (NIHSS or infarct volume), cortisol is the only independent predictor of neutrophilia and lymphopenia.

Of the 114 patients enrolled in the study, 7 patients developed an infection in the first 3 days after stroke and 1 patient died in the week after presentation. Of the remaining 106 patients, 22 patients (21%) developed an infection by day 15.[5] "Clinically abnormal" cell counts at day 3 were not independently predictive of infection (Table 3). For the 94 patients for whom 3-month follow-up was available, 20 patients (21%) had poor outcome (mRS > 3), and lymphopenia at day 3 was independently predictive of poor outcome at day 90 after stroke (Table 3).

Discussion

Changes in leukocyte numbers after stroke are well described. Most studies, however, tend to look at changes in cell counts as a continuous variable, yet the clinical implications of changes within the normal clinical range