Temporal Trends in the Levels of Peripherally Circulating Leukocyte Subtypes in the Hours after Ischemic Stroke

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Background: Leukocyte-mediated neuroinflammation may affect outcomes after ischemic stroke. *Aims:* To explore temporal changes in levels of peripherally circulating leukocyte subtypes in the early hours after ischemic stroke in humans. *Methods:* Retrospective analysis of a single-center database of consecutive thrombolysis cases for acute ischemic stroke (AIS). Multivariable regression analysis was used to explore temporal changes in the levels of peripherally circulating leukocyte subtypes in the hours immediately after ischemic stroke. A natural logarithm transformation was used to achieve normally distributed residuals, and adjustment was made for the severity of stroke, blood glucose concentration, sex, and age. *Results:* Blood samples were taken a median time of approximately 2 hours after stroke symptom onset. Median peripheral neutrophil and lymphocyte counts on admission were 4.8×10^9cells per liter (interquartile range [IQR], 3.6-7.2 $\times 10^9$ cells per liter) and 1.9×10^9cells per liter (IQR, 1.3-2.6 $\times 10^9$cells per liter), respectively. Multivariable regression analysis revealed that after adjustment there was a linear increase in the natural logarithm of the peripheral neutrophil count ($P < .01$), with a linear decrease in the natural logarithm of the peripheral lymphocyte count ($P < .01$) in the hours immediately after stroke onset. No significant temporal associations were found between the levels of the other peripherally circulating leukocyte subtypes. *Conclusions:* Immediately after ischemic stroke, there is an exponential increase in the neutrophil count and an exponential decrease in the lymphocyte count. **Key Words:** Ischemic stroke—neuroinflammation—inflammation—leukocyte—neutrophil—lymphocyte.

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Introduction

Ischemic stroke is a leading cause of adult disability and mortality worldwide.[1] Neuroinflammation has been identified as a key element in the cascade that follows, and recent studies have suggested that systemic inflammation contributes to the etiology and further development of acute ischemic stroke (AIS).[2,3] Leukocytes have been postulated as mediators in the pathogenesis of AIS.

Leukocytosis in the context of AIS is a poor prognostic factor, correlating with increased mortality, lack of neurological improvement, and prolonged hospitalization.[2-5] Neutrophils are the first leukocyte subtype to show substantial upregulation in gene expression following AIS and contribute to cerebral edema and the disruption of the blood–brain barrier.[3,4] Recruitment of neutrophils into the brain has been detected as early as 5 hours after stroke onset and peaks at 24-48 hours in experimental models.[5] Early neutrophil activation has been implicated in the potentiation of postischemic brain injury and correlates with increased stroke severity and a worse functional outcome.[4] Furthermore, some experimental studies have suggested that depleting circulating neutrophils either before or at the onset of stroke reduces infarct volume.[6] Lymphopenia and monocytosis also have been shown to correlate with infarct volume in the setting of AIS.[7] Moreover, distinct monocyte subtypes are associated with complications during the acute and subacute phases of ischemic stroke.[8] However, relatively little is known about trends in peripherally circulating leukocyte levels in the hours immediately after ischemic stroke onset.[9]

In this work, we investigated the early kinetics of peripheral leukocyte subsets in a retrospective cohort study of patients admitted within hours of ischemic stroke onset.

Methods

Population

This was a retrospective cohort study of patients treated with intravenous recombinant tissue plasminogen activator (rtPA) at the Imperial College Healthcare NHS Trust between October 1, 2011, and June 30, 2015, inclusive. Cases that were subsequently deemed to have a nonstroke diagnosis (following a review of neuroimaging and subsequent clinical course by 1 or more consultant stroke physicians) were excluded from analysis. Mechanical thrombectomy cases varied in their time course as compared with patients undergoing rtPA thrombolysis and were therefore also excluded.

Clinical Assessment

The following clinical characteristics were analyzed in the patient cohort: duration of time elapsed from symptom onset to measurement of peripheral leukocyte count (hours), clinical stroke severity on presentation measured using the National Institutes of Health Stroke Scale (NIHSS), capillary blood glucose (mmol/L) on presentation, sex, and age. All recording clinicians had received suitable training in using the NIHSS for the assessment of stroke severity.

Blood Sampling

All patients underwent routine peripheral venous blood sampling for full blood count (to include total leukocyte, neutrophil, monocyte, basophil, eosinophil, and lymphocyte counts, measured as cells $\times 10^9$ per liter) and C-reactive protein (CRP) either prior to or following the administration of rtPA.

Statistical Analysis

Statistical analysis was performed using Stata 14 (StataCorp LP, College Station, TX). Summary statistics for the cohort are offered as percentages, with medians and interquartile ranges (IQRs) given for nonparametric data. Multivariable regression analysis was used to explore temporal changes in the natural logarithmic transformation of levels of peripherally circulating leukocyte subtypes after stroke, making adjustment for the severity of stroke (as measured by the NIHSS), blood capillary glucose concentration, sex, and age. The levels of peripherally circulating leukocyte subtypes underwent a natural logarithm transformation to ensure a normal distribution of residuals after multivariable regression, in keeping with the assumptions of this model. Sensitivity analysis to exclude cases with evidence of inflammation prior to stroke onset was performed by removing cases where CRP levels were greater than 5 mg/L at the time of blood sampling. A Bonferroni correction was used for multiple testing, with a P value less than .01 therefore used to demonstrate statistical significance.

Ethical Review

Only anonymized data already acquired for service evaluation purposes were used in this work. The study proposal was reviewed and approved locally, and further ethical evaluation was not deemed necessary.

Results

Between October 1, 2011, and June 30, 2015, inclusive, a total of 535 patients were consecutively treated with thrombolysis for AIS at the Imperial College Healthcare NHS Trust. Of these, 46 thrombectomy cases and 50 patients later confirmed to have a nonstroke diagnosis were excluded, leaving a total cohort of 439 patients.

Of the 439 eligible patients, complete data on leukocyte count, NIHSS score on hospital presentation, capillary blood glucose on presentation, sex, and age were available for 326 patients. Missing data were attributed to lack