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Peripheral Monocyte Count is Associated with Case-Fatality after Intracerebral Hemorrhage

Opeolu Adeoye, MD MS^{a,b,c}, Kyle Walsh, MD^b, Jessica G. Woo, PhD^d, Mary Haverbusch, RN BSN^e, Charles J. Moomaw, PhD^e, Joseph P. Broderick, MD^{a,e}, Brett M. Kissela, MD MS^{a,e}, Dawn Kleindorfer, MD^{a,e}, Matthew L. Flaherty, MD^{a,e}, and Daniel Woo, MD MS^{a,e}

^aUC Neuroscience Institute, Cincinnati, OH 45267, USA

^bDepartment of Emergency Medicine, University of Cincinnati, Cincinnati, OH, USA

^cDepartment of Neurosurgery, University of Cincinnati, Cincinnati, OH, USA

^dCincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

^eDepartment of Neurology, University of Cincinnati, Cincinnati, OH, USA

Abstract

Background—Leukocytosis is associated with hemorrhage volume and early neurological deterioration after intracerebral hemorrhage (ICH). We examined total white blood cell (WBC) count and absolute monocyte (AMC) and neutrophil (ANC) counts as potential readily available prognostic biomarkers in human ICH.

Methods—In a retrospective study, adult patients aged ≥ 18 years who presented to one of two local hospitals with nontraumatic ICH from July 2008 to December 2009 within 12 hours of symptom onset were identified. Demographics, Glasgow coma scale (GCS), ICH volume, ICH location, and 30-day case-fatality rates were determined. Total WBC count, ANC, AMC, and hemoglobin concentration were determined. Linear and logistic regression were used to evaluate factors associated with baseline ICH volume (log transformed) and 30-day case-fatality, respectively.

Results—Of the 186 patients, mean (±SD) age was 67.3±14.8 years, 51% were male, 22% were black. Median [IQR] ICH volume was 12.8 [4.9, 29.4] ml. After adjusting for patient age and initial hemoglobin, higher initial WBC count (p=0.0009) and higher ANC (p=0.006) were associated with higher ICH volume, whereas AMC was not (p=0.4). After adjusting for patient age, GCS, intraventricular hemorrhage (+/−), stroke location, and ICH volume, baseline AMC was associated with greater odds of 30-day case-fatality (OR 2.26, 95% CI 1.10–4.65, p=0.03).

Conclusion—The association of AMC with higher 30-day case-fatality after ICH is hypothesis generating. Given the lack of association between presenting AMC and ICH volume, AMC may contribute to secondary injury after ICH (hematoma expansion and/or cerebral edema).

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Corresponding Author: Opeolu Adeoye, MD MS, Division of Neurocritical Care, Departments of Emergency Medicine and Neurosurgery, University of Cincinnati, 231 Albert Sabin Way, ML 0769, Cincinnati, OH 45267, Phone: 513-558-3117, Fax: 513-558-5791, opeolu.adeoye@uc.edu.

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Conflicts of interest

All authors report no conflicts of interest

Keywords

Hemorrhagic stroke; inflammation; intracerebral hemorrhage; monocyte; neutrophil; stroke

Introduction

Intracerebral hemorrhage (ICH) occurs in about 70,000 people a year in the United States and has a 30-day mortality of 32–50%.^(1, 2) The strongest contributors to this high mortality are the initial size of the hematoma, whether the hematoma continues to expand after presentation, and whether delayed cerebral edema occurs.^(3–5) To date, no medical or surgical clinical trials have demonstrated a definitive functional outcome benefit after ICH. Thus, ICH remains a challenging clinical and public health problem.

The pathophysiology of ICH remains poorly understood. However, it is well recognized that infiltrating white blood cells (WBC) play a role in secondary injury after ICH.^(6, 7) Preclinical studies have found that leukocytes are present in and around the hematoma within hours after ICH.⁽⁸⁾ Neutrophils infiltrate the ICH first and may cause direct injury by releasing reactive oxygen species, inflammatory proteases and damage to the blood brain barrier.^(6, 7) Neutrophils also facilitate the recruitment of monocytes to the ICH site, and neutrophil depletion has been associated with reduced monocyte infiltration and improved outcome in mouse models of ICH.⁽⁹⁾ Toll-like receptor (TLR) 4 mediates the recruitment of monocytes and inflammatory monocytes to the ICH site, and TLR 4 deficient mice were found to have improved outcome after ICH compared with wild type animals.⁽¹⁰⁾

In clinical studies, the total peripheral WBC count has been associated with baseline hemorrhage volume, early neurological deterioration and functional outcomes after ICH.^(11–14) Neutrophils and monocytes are predominant WBCs and peripheral neutrophil and monocyte infiltration have been found to contribute to secondary injury in animal models of ICH.^(15, 16) Monocytes in the blood stream play a central role in the pathogenesis of atherosclerosis and cardiovascular disease.⁽¹⁷⁾ Circulating monocytes also express significant amounts of tissue factor, interact with platelets, and modulate thrombosis and hemostasis.^(18–21) A recent clinical study found that increased expression of TLR 2 and TLR 4 in monocytes was associated with poor outcome after ICH.⁽²²⁾

If peripheral monocyte and neutrophil counts, readily available biomarkers, are found to be strongly predictive of hematoma expansion, cerebral edema and/or ICH outcome it would lead to targeted research questions that may generate new treatments in ICH patients with elevated WBC counts. In this report, we determined the association of peripheral WBC, monocyte and neutrophil counts with baseline ICH volume and 30-day case-fatality. We hypothesized that total WBC, absolute monocyte and neutrophil counts at presentation and change in monocyte and neutrophil counts from presentation to 24 hours will be associated with presenting ICH volume and 30-day case-fatality.

Materials and Methods

We analyzed a group of patients aged 18 or older with ICH previously identified as part of the Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS) study from July 2008 to December, 2009. The institutional review board for each participating hospital system approved the GERFHS study. Cases were identified by retrospective review of primary and secondary ICD-9 codes 430 to 438.9 at all 16 acute care hospitals in the 5-county Greater Cincinnati/Northern Kentucky region. Study nurses also maintained active surveillance (“hot pursuit”) at several hospitals that treat most ICH in the area. All potential

cases were abstracted by study nurses and reviewed in detail by study physicians. Exclusion criteria were previous ICH, traumatic ICH, hemorrhagic cerebral infarction, and hemorrhage associated with brain tumor, encephalitis, recent endarterectomy, and thrombolytic treatment of ischemic stroke. Patient demographics and putative risk factors for ICH were recorded by chart review. Full details of the GERFHS methodology have been published elsewhere.(23)

For this project, we included GERFHS patients seen at the two hospitals in the region with the largest number of ICH patients.(24) Patients were further selected to exclude those whose symptom onset occurred more than 12 hours before the timing of baseline data. We then performed additional chart review to determine baseline and 24 hour total WBC, absolute monocyte and absolute neutrophil counts. Absolute monocyte and neutrophil counts were calculated by multiplying the total WBC count by reported percent monocyte or neutrophil as appropriate. These values are routinely reported in hospital laboratories with the Complete Blood Count (CBC), a ubiquitous lab in hospitalized patients. While the initial chart review for GERFHS recorded WBC counts, the monocyte and neutrophil counts were not recorded. Demographics, ICH volumes, hematoma expansion and other clinical parameters of interest were previously determined as part of the GERFHS study.

Statistical analysis was conducted using SAS v.9.3 (SAS Institute, Cary, NC), and monocyte, neutrophil and WBC counts and ICH volume were log transformed to correct for non-normality. The association of ICH volume with WBC counts was determined using linear regression. The association of WBC counts with 30-day case-fatality was determined using logistic regression. For both linear and logistic regression, sets of covariates were identified *a priori* and individual covariates were retained in the final model if significant ($p < 0.05$).

Results

We identified 186 ICH patients seen in the ED within 12 hours of symptom onset and with complete baseline data. Mean age was 67.3 ± 14.8 years; 51% were male, and 22% black. Median [interquartile range] ICH volume was 12.8mL [4.9, 29.4]. Demographics and other clinical information are presented in Table 1.

After adjusting for patient age and initial hemoglobin (since anemia has been associated with larger ICH volume) (11), higher initial WBC count ($p = 0.0009$) and higher neutrophil count ($p = 0.006$) were significantly associated with larger baseline ICH volume, but monocyte count was not (Table 2). After adjusting for patient age, Glasgow coma scale (GCS) score, the presence or absence of intraventricular hemorrhage, hemorrhage location, and ICH volume, the initial monocyte count was independently associated with greater odds of 30-day mortality (2.26-fold increased odds for every log-transformed unit of monocytes) (Table 3).

We examined whether the time from symptom onset to blood draw would impact the monocyte and neutrophil counts obtained. A longer time between symptom onset and initial blood draw was associated with lower monocytes ($p = 0.03$) and higher neutrophils ($p = 0.01$). We also examined the changes in WBC early in the course of ICH. A repeat CBC with available monocyte and neutrophil counts was obtained 6.1 ± 3.8 hours after the baseline labs in 73 patients. A longer time between initial and repeat blood draws was associated with greater changes in neutrophil count during this period ($p = 0.03$), which suggests that neutrophils may continue to rise after initial blood draw, but no other time associated changes were observed. The early changes in these counts were not associated with baseline ICH volume or 30-day case-fatality. Of the 186 patients included, 59 were on aspirin alone, 15 were on warfarin alone and 31 were on both; 81 patients were not on either warfarin or

aspirin. Adjusting for warfarin or aspirin use did not impact the overall models. Follow up hematoma volumes on CTs done within 24 hours of the baseline CT were available on 89 out of 186 subjects. Among these, 62% (n=55) experienced hematoma expansion (defined as any increase in hematoma volume) and 38% (n=34) did not. We found no association of baseline WBC, ANC or AMC with hematoma expansion. Adjusting for onset time to baseline CT, baseline ICH volume, hemorrhage location, warfarin and/or aspirin use did not change the findings.

Discussion

Our main hypothesis was that absolute monocyte and neutrophil counts at presentation and change in monocyte and neutrophil counts from presentation to 24 hours would be associated with presenting ICH volume and 30-day case-fatality in ICH patients who presented within 12 hours of symptom onset. The basis for this hypothesis was that the absolute number of monocytes available early in the clinical course may allow for more monocytes to be recruited to the site of ICH and thereby contribute to secondary injury after ICH, as has been found in animal models of the disease.(9) We found that higher monocyte count at presentation was independently associated with greater odds of 30-day case-fatality (2.26-fold increased odds for every log-transformed unit of monocytes), adjusting for many confounding factors, including ICH volume and GCS score. Coupled with the fact that we did not find an association between presenting monocyte count and ICH volume, this suggests that the association of monocyte count with case-fatality may be due to the contribution of monocytes to secondary injury after ICH. This novel finding may enhance the understanding of the pathophysiology and clinical course of ICH and lead to further investigations if confirmed in an independent data set.

Secondary injury after ICH may result from hematoma expansion or cerebral edema. While we found no association of monocyte count with hematoma expansion in this report, adequately powered prospective studies are required to investigate the relationship of monocyte count and ICH case-fatality. Barring any obvious link of monocyte count to hematoma expansion and/or cerebral edema, mechanisms by which monocytes may contribute to poor outcome and case-fatality after ICH include increased availability of monocytes to attach to the vascular endothelium thereby damaging the blood brain barrier; increased availability of monocytes to bind to monocyte chemoattractant proteins that are expressed in cerebral vessels and known to facilitate neuronal death and promote vessel injury after monocyte binding(25, 26); and, total expression of TLRs 2 and 4 (among other pro-inflammatory molecules) contributing to cerebral edema and poor outcome.(22)

As has been previously reported,(11,12) we found that higher initial WBC count and higher neutrophil count were significantly associated with larger baseline ICH volume. In contrast to previous reports, these were not independently associated with outcome after we adjusted for monocyte counts. We did not find any associations between early changes in WBC, neutrophil or monocyte counts and ICH volume or case-fatality. However, these results may be due to small sample size, and data for only very early repeat labs were available for comparison.

Limitations of our study include the prolonged time from symptom onset to blood draws. Since hematoma expansion, a strong predictor of ICH case-fatality, primarily occurs within the first 6 hours, the longer interval from symptom-onset to blood draw may have confounded our analyses of factors associated with higher case-fatality.

Conclusion

Overall, neutrophils have been primarily implicated as the inflammatory cells most likely to contribute to secondary injury after ICH, and monocytes have been thought to be recruited to the site of injury primarily as phagocytic cells aiming to limit injury mediated by neutrophils. Recent preclinical studies and now our clinical study suggest that monocytes may have an independent role in affecting ICH outcomes. Despite the limitations discussed above, the association of AMC with higher 30-day case-fatality after ICH is hypothesis generating. Absolute monocyte count, a readily available and ubiquitous biomarker at presentation in ICH patients, may represent a novel prognostic tool after ICH.

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Table 1

Demographics

N	186
Age (mean $\pm$ SD)	67.3 $\pm$ 14.8
Sex (% male)	94 (50.5%)
Race (% white)	146 (78.5%)
Location of stroke	104 (56%) Deep 47 (25%) Lobar 7 (4%) Brainstem 27 (14.5%) Cerebellum 1 (0.5%) Primary intraventricular hemorrhage
Intraventricular hemorrhage (% any)	100 (53.8%)
Died within 30 days (%)	74 (39.8%)
Discharge Modified Rankin Classification (% 0–2)	16 (8.6%)
Baseline GCS (median [IQR])	14 [10, 15]
Baseline ICH volume, mL (median [IQR])	12.8 [4.9, 29.4]

Table 2

Association of Baseline Cell Counts With Baseline ICH Volume*

	$\beta \pm SE$	p-value
WBC (log)	0.97 ± 0.29	0.0009
Monocytes (log)	0.18 ± 0.19	0.4
Neutrophils (log)	0.62 ± 0.22	0.006

* Adjusted for age and baseline hemoglobin

Table 3

Odds Ratios for 30-day Case-Fatality*

	OR (CI)	p-value
WBC (log)	1.2 (0.38, 3.68)	0.8
Monocytes (log)	2.26 (1.10, 4.65)	0.03
Neutrophils (log)	0.88 (0.38, 2.04)	0.8

* Adjusted for age, GCS, presence of intraventricular hemorrhage, hemorrhage location, and ICH volume