Monocyte Count and 30-Day Case Fatality in Intracerebral Hemorrhage

Kyle B. Walsh, MD; Padmini Sekar, MS; Carl D. Langefeld, PhD; Charles J. Moomaw, PhD; Mitchell S.V. Elkind, MD; Amelia K. Boehme, PhD, MSPH; Michael L. James, MD; Jennifer Osborne, BSN; Kevin N. Sheth, MD; Daniel Woo, MD, MS; Opeolu Adeoye, MD, MS

Background and Purpose—Monocytes may contribute to secondary injury after intracerebral hemorrhage (ICH). We tested the association of absolute monocyte count with 30-day ICH case fatality in a multiethnic cohort.

Methods—Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study is a prospective, multicenter, case–control study of ICH among white, black, and Hispanic patients. In 240 adults with nontraumatic ICH within 24 hours of symptom onset, we evaluated the influence of ICH score and complete blood count components on 30-day case fatality using generalized linear models.

Results—Mean age was 62.8 years (SD, 14 years); 61.7% were men, 33.3% black, and 29.6% Hispanic. Median ICH volume was 9.9 mL (interquartile range, 4.4–26.7). After adjusting for patient age and initial hemoglobin, higher total white blood cell count (P=0.0011), driven by higher absolute neutrophil count (P=0.002), was associated with larger ICH volume, whereas absolute monocyte count was not (P=0.15). After adjusting for age, Glasgow Coma Scale, ICH volume, location, and the presence or absence of intraventricular hemorrhage, baseline absolute monocyte count was independently associated with higher 30-day case-fatality (odds ratio, 5.39; 95% confidence interval, 1.87–15.49; P=0.0018), whereas absolute neutrophil count (odds ratio, 1.04; 0.46–2.32; P=0.93) and white blood cell count (odds ratio, 1.62; 0.58–4.54; P=0.36) were not.

Conclusions—These data support an independent association between higher admission absolute monocyte count and 30-day case-fatality in ICH. Inquiry into monocyte-mediated pathways of inflammation and apoptosis may elucidate the basis for the observed association and may be targets for ICH neuroprotection. **(*Stroke*. 2015;46:2302-2304. DOI: 10.1161/STROKEAHA.115.009880.)**

Key Words: cerebral hemorrhage ■ Glasgow Coma Scale ■ inflammation ■ monocytes ■ odds ratio

Intracerebral hemorrhage (ICH) accounts for 10% of all strokes but 50% of stroke mortality.[1,2] No therapies have shown definitive benefit after ICH. Infiltrating white blood cells (WBCs) play a role in secondary injury after ICH.[3] In clinical studies, WBC count has been associated with larger ICH volume,[4] early neurological deterioration,[5,6] and worse discharge disposition.[7] However, the individual contributions of leukocyte cell types remain unclear.

In a murine ICH study, circulating inflammatory monocytes outnumbered other leukocytes in brain tissue, and mice with fewer inflammatory monocytes had better motor function.[8] Limiting monocyte recruitment into brain tissue after ICH also resulted in less neurobehavioral disability.[9] A clinical study of 85 ICH patients found that higher serum monocyte chemoattractant protein-1, the dominant chemokine for monocyte recruitment, at 24 hours was independently associated with worse modified Rankin Scale at 7 days.[8] Based on these data, we recently investigated associations between absolute monocyte count (AMC), ICH volume, and 30-day fatality in 186 ICH patients who presented within 12 hours of symptom onset. AMC was not associated with ICH volume, but was independently associated with case-fatality.[10]

In the present study, we seek to confirm our prior findings using a cohort independent of the discovery set of ICH patients in a multiethnic, multicenter study, by determining the association of WBC count, absolute neutrophil count (ANC), and AMC with baseline ICH volume and 30-day case-fatality.

Methods

Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study is a prospective, multicenter, case–control study of ICH among white, black, and Hispanic patients. The methods of the ERICH study have

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From the Department of Emergency Medicine (K.B.W., O.A.) and Department of Neurology and Rehabilitation Medicine (P.S., C.J.M., J.O., D.W.), University of Cincinnati (UC), OH; Department of Biostatistics, Center for Public Health Genomics, Wake Forest School of Medicine, Winston-Salem, NC (C.D.L.); Department of Neurology, College of Physicians and Surgeons, and Epidemiology, Mailman School of Public Health, Columbia University, New York, NY (M.S.V.E., A.K.B.); Departments of Anesthesiology and Neurology, Duke University, Durham, NC (M.L.J.); Department of Neurology, Yale University, New Haven, CT (K.N.S.); and UC Neuroscience Institute, OH (D.W., O.A.).

Correspondence to Opeolu Adeoye, MD, MS, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267-0769. E-mail Opeolu.Adeoye@uc.edu

Table 1. Characteristics of Included Ethnic/Racial Variations of Intracerebral Hemorrhage Patients

n	240
Age, mean (SD), y	62.8 (14.0)
Sex (% male)	148 (61.7)
Race categories, n (%)	
Black	80 (33.3)
Hispanic	71 (29.6)
White	89 (37.1)
Location	
Deep	147 (61.3)
Lobar	62 (25.8)
Brain stem	10 (4.2)
Cerebellum	21 (8.8)
Any IVH, n (%)	70 (29.2)
Dead at discharge, n (%)	29 (12.1)
Dead at 30 days, n (%)	38 (15.8)
Discharge mRS 0–2, n (%)	53 (22.1)
Baseline GCS, median (IQR)	14 (11, 15)
Baseline ICH volume Geometric mean (95% CI)	10.7 (9.2–12.5)

CI indicates confidence interval; GCS, Glasgow Coma Scale; ICH, intracerebral hemorrhage; IQR, interquartile range; IVH, intraventricular hemorrhage; and mRS, modified Rankin Scale.

been published previously.[2] Briefly, self-reported non-Hispanic white, non-Hispanic black, and Hispanic ICH patients aged ≥18 years, resident within 75 miles of 1 of the 19 recruitment centers (within 100 miles for population centers <1 million), with spontaneous ICH and informed consent provided by the patient/legal representative were included. ICH was defined as a spontaneous, nontraumatic, abrupt onset of severe headache, altered level of consciousness, or focal neurological deficit that is associated with focal blood collection within brain parenchyma (including peripartum and warfarin-associated ICH) seen on neuroimaging. Cases of ICH because of malignancy-associated coagulopathy, dural venous sinus thrombosis, vascular malformations, aneurysms, tumors, or hemorrhagic conversion of a recent ischemic stroke were excluded.

Demographics, Glasgow Coma Scale score, 30-day case-fatality, WBC, and hemoglobin concentration were among the items recorded on case report forms, and ICH volume was determined by the central imaging core. Additional data were required for the present study; ANC and AMC were obtained for a sample of 240 patients whose initial laboratory studies were completed within 24 hours of symptom onset. The periods of enrollment varied by recruitment center; overall, ranging from November 2010 through December 2013. Linear regression was used to test for an association with ICH volume (natural log transformed) and logistic regression for factors associated with 30-day case-fatality. Regression diagnostics were computed to examine model fit to these data. To minimize the influence of extreme values of predictors on the model, WBC, ANC, and AMC were natural log transformed.

Results

Table 1 shows the characteristics of included patients. After adjusting for patient age and initial hemoglobin, higher total WBC count (P=0.0011), driven by higher ANC (P=0.002), was associated with larger ICH volume, whereas AMC was not (P=0.15; Table 2). Odds ratios for 30-day case-fatality were determined after adjusting for age, Glasgow Coma Scale, ICH volume, ICH location, and the presence or absence of intraventricular hemorrhage. Higher baseline AMC was independently associated with 30-day case-fatality (odds ratio, 5.39; 95% confidence interval, 1.87–15.49; P=0.0018), whereas ANC (odds ratio, 1.04; 0.46–2.32; P=0.93) and total WBC count (odds ratio, 1.62; 0.58–4.54; P=0.36) were not (Table 3).

Discussion

We confirmed an independent association of AMC with 30-day ICH case-fatality after adjusting for important confounders.[11] Our present findings are consistent with prior data.[10] Associations of higher WBC count and ANC with ICH volume have been reported by other investigators, but likely represent an acute phase response.[4,7] Our initial report was the first to suggest an independent role for monocytes.[10] Confirming those findings provides additional support for the concept of monocytes specifically contributing to secondary injury after ICH. Proposed mechanisms include damage to the blood–brain barrier, binding to chemoattractant proteins in cerebral vessels that promotes neuronal death and cell injury,[12] and contribution to cerebral edema.[13]

The therapeutic implication of our findings is that monocyte inflammatory pathways may be targets for neuroprotection in ICH. Preclinical models suggest that monocyte depletion,[8] reduction in monocyte recruitment to the site of ICH, and targeted antibodies to reduce monocyte infiltration[9] may all result in improved functional outcome after ICH. Our findings provide justification for well-designed preclinical and early phase clinical studies investigating the inhibition of inflammatory monocytes in ICH. In ischemic stroke, preclinical evidence has led to a clinical study investigating natalizumab, a monoclonal antibody that blocks leukocyte adhesion to endothelial cells and is approved for the treatment of multiple sclerosis, for reducing infarct size.[14] Thus, natalizumab or similar agents may be candidates for preclinical and clinical studies in ICH. Recent data also suggest the interaction of initial monocyte expansion and subsequent suppression via the HMGB1-RAGE (high mobility group box 1–receptor for advanced glycation end products) pathway may influence observed outcomes in ischemic stroke.[15] This line of inquiry may further elucidate targets for intervention.

Limitations of our study include its retrospective nature, inability to investigate temporal trends in cell counts, and lack of follow-up imaging data allowing for investigation of the association of leukocyte counts and hematoma expansion. Our prior report found no association of AMC with hematoma expansion.[10]

Conclusions

Our findings complement a growing body of evidence from clinical and preclinical investigations supporting a unique role

Table 2. Association of Baseline Cell Counts With Baseline Intracerebral Hemorrhage Volume*

	β	SE	*P* Value
Total WBC (log)	0.53	0.16	0.0011
Neutrophils (log)	0.38	0.12	0.0020
Monocytes (log)	0.18	0.13	0.15

WBC indicates white blood cell.
*Adjusted for age and baseline hemoglobin.

Table 3. Association of Leukocyte Counts With 30-Day Case Fatality

	Total WBC (Log) OR (95% CI)	Total WBC (Log) *P* Value	Neutrophils (Log) OR (95% CI)	Neutrophils (Log) *P* Value	Monocytes (Log) OR (95% CI)	Monocytes (Log) *P* Value
Unadjusted	3.24 (1.50–7.00)	0.0028	2.13 (1.16–3.90)	0.014	3.40 (1.64–7.02)	0.0010
Adjusted*	1.62 (0.58–4.54)	0.36	1.04 (0.46–2.32)	0.93	5.39 (1.87–15.49)	0.0018
Covariates						
Age, y	1.09 (1.05–1.14)	<0.0001	1.09 (1.05–1.14)	<0.0001	1.11 (1.06–1.16)	<0.0001
GCS	0.84 (0.74–0.96)	0.0086	0.82 (0.73–0.94)	0.0026	0.84 (0.74–0.96)	0.0083
log(1+volume)	4.30 (2.3–8.2)	<0.0001	4.30 (2.3–8.0)	<0.0001	5.10 (2.6–10.0)	<0.0001
Location: deep	7.7 (2.0–30.2)	0.0034	7.3 (1.9–28.3)	0.0039	10.2 (2.5–41.5)	0.0011
Location: infratentorial	11.7 (1.7–80.1)	0.012	13.0 (1.9–88.9)	0.0089	13.7 (1.8–104.7)	0.012
Presence of IVH	0.5 (0.2–1.5)	0.24	0.5 (0.2–1.5)	0.24	0.5 (0.2–1.4)	0.19

CI indicates confidence interval; GCS, Glasgow Coma Scale; IVH, intraventricular hemorrhage; OR, odds ratio; and WBC, white blood cell count.

*Adjusted for age, GCS, intracerebral hemorrhage volume, location, and presence of IVH.

of monocytes in secondary neuroinflammatory injury after ICH. Inhibitors of monocyte migration and activity may be novel therapeutic targets for ICH.

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Disclosures

None.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation*. 2015;131:e29–322. doi: 10.1161/CIR.0000000000000152.
2. Woo D, Rosand J, Kidwell C, McCauley JL, Osborne J, Brown MW, et al. The Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study protocol. *Stroke*. 2013;44:e120–e125. doi: 10.1161/STROKEAHA.113.002332.
3. Wang J. Preclinical and clinical research on inflammation after intracerebral hemorrhage. *Prog Neurobiol*. 2010;92:463–477. doi: 10.1016/j.pneurobio.2010.08.001.
4. Kumar MA, Rost NS, Snider RW, Chanderraj R, Greenberg SM, Smith EE, et al. Anemia and hematoma volume in acute intracerebral hemorrhage. *Crit Care Med*. 2009;37:1442–1447. doi: 10.1097/CCM.0b013e31819ced3a.
5. Sun W, Peacock A, Becker J, Phillips-Bute B, Laskowitz DT, James ML. Correlation of leukocytosis with early neurological deterioration following supratentorial intracerebral hemorrhage. *J Clin Neurosci*. 2012;19:1096–1100. doi: 10.1016/j.jocn.2011.11.020.
6. Leira R, Dávalos A, Silva Y, Gil-Peralta A, Tejada J, Garcia M, et al; Stroke Project, Cerebrovascular Diseases Group of the Spanish Neurological Society. Early neurologic deterioration in intracerebral hemorrhage: predictors and associated factors. *Neurology*. 2004;63:461–467.
7. Agnihotri S, Czap A, Staff I, Fortunato G, McCullough LD. Peripheral leukocyte counts and outcomes after intracerebral hemorrhage. *J Neuroinflammation*. 2011;8:160. doi: 10.1186/1742-2094-8-160.
8. Hammond MD, Taylor RA, Mullen MT, Ai Y, Aguila HL, Mack M, et al. CCR2+ Ly6C(hi) inflammatory monocyte recruitment exacerbates acute disability following intracerebral hemorrhage. *J Neurosci*. 2014;34:3901–3909. doi: 10.1523/JNEUROSCI.4070-13.2014.
9. Hammond MD, Ambler WG, Ai Y, Sansing LH. α4 integrin is a regulator of leukocyte recruitment after experimental intracerebral hemorrhage. *Stroke*. 2014;45:2485–2487. doi: 10.1161/STROKEAHA.114.005551.
10. Adeoye O, Walsh K, Woo JG, Haverbusch M, Moomaw CJ, Broderick JP, et al. Peripheral monocyte count is associated with case fatality after intracerebral hemorrhage. *J Stroke Cerebrovasc Dis*. 2014;23:e107–e111. doi: 10.1016/j.jstrokecerebrovasdis.2013.09.006.
11. Hemphill JC 3rd, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH score: a simple, reliable grading scale for intracerebral hemorrhage. *Stroke*. 2001;32:891–897.
12. Conductier G, Blondeau N, Guyon A, Nahon JL, Rovère C. The role of monocyte chemoattractant protein MCP1/CCL2 in neuroinflammatory diseases. *J Neuroimmunol*. 2010;224:93–100. doi: 10.1016/j.jneuroim.2010.05.010.
13. Rodríguez-Yáñez M, Brea D, Arias S, Blanco M, Pumar JM, Castillo J, et al. Increased expression of Toll-like receptors 2 and 4 is associated with poor outcome in intracerebral hemorrhage. *J Neuroimmunol*. 2012;247:75–80. doi: 10.1016/j.jneuroim.2012.03.019.
14 *Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke*. U.S. National Institutes of Health ClinicalTrials.gov website. https://www.clinicaltrials.gov/ct2/show/record/NCT01955707. Accessed April 7, 2015.
15. Liesz A, Dalpke A, Mracsko E, Antoine DJ, Roth S, Zhou W, et al. DAMP signaling is a key pathway inducing immune modulation after brain injury. *J Neurosci*. 2015;35:583–598. doi: 10.1523/JNEUROSCI.2439-14.2015.

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