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# Monocyte count and 30-day case-fatality in intracerebral hemorrhage

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## Abstract

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

**Methods—**Ethnic/Racial Variations of Intracerebral Hemorrhage (ERICH) study is a prospective, multi-center, case-control study of ICH among white, black, and Hispanic patients. In 240 adults with non-traumatic 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 14years); 61.7% were male, 33.3% black, and 29.6% Hispanic. Median ICH volume was 9.9ml (IQR 4.4–26.7). After adjusting for patient age and initial hemoglobin, higher total white blood cell count (WBC) (p=0.0011), driven by higher absolute neutrophil count (ANC) (p= 0.002), was associated with larger ICH volume, whereas absolute monocyte count (AMC) was not (p=0.15). After adjusting for age, GCS, ICH volume, location, and presence or absence of intraventricular hemorrhage, baseline AMC was independently associated with higher 30-day case-fatality (OR 5.39, 95%CI 1.87–15.49,

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p=0.0018) whereas ANC (OR 1.04, 0.46-2.32, p=0.93) and WBC (OR 1.62, 0.58–4.54, p=0.36) were not.

**Conclusions—**These data support an independent association between higher admission AMC 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.

### Keywords

intracerebral hemorrhage; case-fatality; monocytes; inflammation

## Introduction

Intracerebral hemorrhage (ICH) accounts for 10% of all strokes but 50% of stroke mortality.[1,2] No therapies have shown definitive benefit following ICH. Infiltrating white blood cells (WBC) play a role in secondary injury after ICH.[3] In clinical studies, WBC count has been associated with larger ICH volume,[4] early neurologic 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 higher serum monocyte chemoattractant protein-1, the dominant chemokine for monocyte recruitment, at 24 hours was independently associated with worse modified Rankin Scale (mRS) at seven 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 utilizing a cohort independent of the discovery set of ICH patients in a multi-ethnic, 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, multi-center, case-control study of ICH among white, black, and Hispanic patients. The methods of the ERICH study have 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 one of the 19 recruitment centers (within 100 miles for population centers less than 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 neurologic deficit that is

associated with focal blood collection within brain parenchyma (including peripartum and warfarin-associated ICH) seen on neuroimaging. Cases of ICH due to 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 (GCS) 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 24hours 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 (OR) for 30-day case-fatality were determined after adjusting for age, GCS, ICH volume, ICH location, and presence or absence of intraventricular hemorrhage (IVH). Higher baseline AMC was independently associated with 30-day case-fatality (OR 5.39, 95%CI 1.87–15.49, p=0.0018), whereas ANC (OR 1.04, 0.46-2.32, p=0.93) and total WBC count (OR 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 following 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 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 suggests the interaction of initial monocyte expansion and subsequent suppression via the HMGB1-RAGE 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]

## Conclusion

Our findings complement a growing body of evidence from clinical and preclinical investigations supporting a unique role of monocytes in secondary neuroinflammatory injury following ICH. Inhibitors of monocyte migration and activity may be novel therapeutic targets for ICH.

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**Table 1**
**Characteristics of Included ERICH Patients**

| | |
|---|---|
| N | 240 |
| Age mean (SD) | 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) |
| - Brainstem | 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) |

## Table 2

**Association of baseline cell counts with baseline ICH volume***

| | Beta | 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 |

*Adjusted for age and baseline hemoglobin

**Table 3**

**Association of leukocyte counts with 30-day case-fatality**

| | Total WBC (log) OR(CI) | Total WBC (log) P-value | Neutrophils (log) OR(CI) | Neutrophils (log) P-value | Monocytes (log) OR(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 | 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 |

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