# An MRI Hyperintense Acute Reperfusion Marker is Related to Elevated Peripheral Monocyte Count in Acute Ischemic Stroke

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## Abstract

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**Background and Purpose—**Blood-brain-barrier (BBB) disruption detected on MRI in acute ischemic stroke as a hyperintense acute reperfusion marker (HARM) is associated with upregulation of matrix metalloproteinase-9 (MMP-9). Although activated leukocytes, including monocytes, are the main source of MMPs, limited data exists to support relationship between leukocyte activation and BBB disruption in patients with acute ischemic stroke. The goal of this study is to investigate the relationship between neutrophils, lymphocytes, and monocytes with BBB disruption detected as HARM (+) in patients with acute ischemic stroke.

**Methods—**We conducted a retrospective analysis of prospectively collected data in patients that did not receive any reperfusion therapy with acute (< 12 hours) ischemic stroke. MRI scans were obtained at baseline, 24 hours, and 5 days. HARM was evaluated on the 24-hour follow-up scan.

**Results—**Thirty-three patients were studied. HARM was detected in 27% of patients. Median volumes of baseline perfusion (mean transit time - MTT) deficit (219.4 mL vs 158.4 mL, p=0.029) and DWI infarct growth at 24 hours (18.50 mL vs. 0.14 mL, p=0.017), as well as the median absolute numbers ($1 \times 10^3/\text{mm}^3$) of monocytes, were significantly higher in HARM (+) versus HARM (−) patients (0.9 vs. 0.6, p=0.011).

**Conclusion—**Increased monocyte count associated with HARM supports importance of systemic inflammation in BBB disruption in acute ischemic stroke.

## Keywords

stroke; blood-brain barrier; monocytes; MRI

## INTRODUCTION

Blood-brain-barrier (BBB) disruption in acute stroke can be detected as enhancement of the cerebrospinal fluid space on post-contrast MRI and has been termed hyperintense acute reperfusion marker (HARM)[1]. Treatment with intravenous t-PA, reperfusion, and advanced age have all been associated with HARM.[1] The most recognized mechanism for acute BBB disruption in stroke is degradation of the basal lamina brought on by upregulation of matrix metalloproteinase (MMP)-9 following the ischemic insult.[1,2] Upregulation of MMPs in acute stroke is a two-step process with MMP-2 being the first enzyme elevated acutely, followed by MMP-9 rise as a second phase of BBB disruption.[2] Although activated leukocytes, including monocytes, are the main source of MMPs,[3] limited data exists to support a causal relationship between leukocyte activation and BBB disruption in patients with acute ischemic stroke. The goal of this pilot study was to investigate the association between elevation of white blood cell count, including neutrophils, lymphocytes or monocytes, and HARM in untreated patients presenting within 12 hours of stroke onset. We hypothesized that elevated white blood cell counts, including neutrophils, lymphocytes and/or monocytes, would be associated with BBB disruption in acute ischemic stroke patients presenting within 12-hours of symptom onset who have not received treatment.

# METHODS

## Patients

We conducted a retrospective analysis of prospectively collected data from the NIH Stroke Natural History Study, which enrolled patients with an admission diagnosis of acute ischemic stroke who were seen by the NIH Stroke Team and screened with MRI between August 1999 and October 31, 2009.[1,4] The appropriate Ethics and Institutional Review Boards (NINDS/NIH IRB for Suburban Hospital, Johns Hopkins Medicine, Bethesda, MD; and Medstar Washington Hospital Center, Washington Hospital Center, Washington, DC IRB) approved the study (NCT00009243).

For the current analysis, consented patients were included if they: (1) were last seen normal 12 hours, (2) had an acute MRI with proven diagnosis of stroke with evaluable diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI), and FLAIR at baseline, 24 hours and 5-day follow-up, and (3) had available baseline blood lab data from the corresponding admission. We excluded patients receiving intravenous (IV) recombinant t-PA (rt-PA) or endovascular therapy as well as patients with hemorrhagic transformation of ischemic stroke. Given the fact that in our previous study IV t-PA was an independent predictor of HARM,[1] to better characterize direct effects of leukocytes on BBB dysfunction, we decided to exclude patients receiving intravenous or endovascular reperfusion therapies. For the purpose of the study, we also excluded patients meeting constitutional criteria for infection (fever > 37.8°C and/or leukocytosis >14,000 leukocytes/mm$^3$), as well as those with history of autoimmune and lymphoproliferative diseases. This is a different cohort of patients which does not overlap with our previously studied HARM population.[1]

Baseline blood for routine clinical labs was obtained in the emergency room within 30 minutes of triage time, and complete blood cell count with differential was analyzed using Beckman Coulter hematology analyzer.

## Image Acquisition

MR imaging was performed at baseline, 24-hours, and 5 days using 1.5T (Twinspeed, General Electric) clinical scanner per previously described protocol.[1,4] Mean transit time (MTT) maps generated from the scanner were used for the baseline perfusion deficit volume measurements. A dosing of 0.1 mmol/kg of Gd-DTPA was administered for PWI.[1]

## Lesion Volume Analysis

The rater reliability statistics for the semi-automated lesion volume analysis with planimetric method used in this study have been published elsewhere.[4] Perfusion-diffusion mismatch (PD MM) was calculated as baseline MTT volume minus baseline DWI volume. Reperfusion volume at 24 hours was calculated as MTT volume at 24-hours minus baseline MTT volume. DWI infarct growth was calculated as DWI volume at 24-hours minus baseline DWI volume.

### HARM Assessment

HARM was evaluated according a previously described method.[1] All FLAIR images were reviewed sequentially in time to allow for the comparison of the baseline scans, performed before administration of Gd-DTPA, with the scans performed 24-hours after Gd-DTPA administration. Presence of HARM (BBB disruption) was identified at 24-hour scan as positive if the cerebrospinal fluid intensity in the sulci, ventricles, background, or vitreous humor appeared hyperintense and continuous across >10 slices. The pre-contrast FLAIR 24-hour scan was used as the criterion for analysis to allow for the maximum accumulation of enhancement. All image interpretations were based on visual inspection performed by four experienced investigators (ZN, ML, RL, LL) who arrived at a consensus reading. First images were interpreted by ZN and ML. In cases of disagreement a third blinded investigator (RL or LL) evaluated resolving disagreement. We did not test the inter- and intra-rater reproducibility. HARM assessment was done blinded to blood data.

### Statistical analysis

Values are reported as mean (±SD), percentage, or median with interquartile range (IQR, 25–75 percentile). Nonparametric tests (Mann Whitney U or Chi-squared) were used to compare the distributions or classifications of variables as appropriate. The software package SPSS 19.0 was used for this analysis.

## RESULTS

Thirty-three patients were studied (Table). Sixty-four percent (21/33) of patients were outside of IV t-PA time window and that was the primary reason they were not treated. For the 12 patients that presented within the time window, the specific excluding factors were not documented with the exception of one patient whose INR>1.7.

Twenty-seven percent of patients had HARM. Baseline MTT lesion volume was significantly larger in patients who were HARM (+) compared to to those who were HARM (−) (219.4 mL vs. 158.4 mL, p=0.029). Baseline median PD MM lesion volume trended to be larger (183.0 mL vs. 129.8 mL, p=0.065) in the HARM (+) group. On 24-hour MRI there was a significant increase in DWI lesion growth in the HARM (+) group compared to the HARM (−) group (18.50 mL vs. 0.14 mL, p=0.017). The median absolute numbers of monocytes were significantly higher in HARM (+) versus HARM (−) patients [0.9 (0.6–1.4) vs. 0.6 (0.4–0.7), p=0.011] (Table).

## DISCUSSION

We found that elevated monocyte count was associated with BBB disruption in untreated stroke patients suggesting a potential contributing role of these cells in the BBB disruption that occurs with acute ischemia.

The most likely explanation for this observation is that activated monocytes express higher levels of proteolytic MMP-9, which might result in BBB disruption and appearance of HARM as was reported in our previous study.[1] Recently, worse stroke clinical outcome was reported in association with elevated pro-inflammatory and proteolytic CD14^{high}CD16^{−}

subpopulation of monocytes.[5] It is likely that tissue damage was mediated through the BBB by pro-inflammatory monocytes, which are the white blood cell population that appears initially in infarcted brain tissue.[6] In a mice model of ischemia/reperfusion, monocytes infiltrated infarcted tissue at 12 hours after cerebral ischemia, while lymphocytes and neutrophils peaked at 3 days.[6]

In this study monocytes, but not neutrophils, were associated with HARM. Although neutrophils, which are the dominant white blood cell line, have MMPs present in an active form, a mouse model of transient focal cerebral ischemia failed to show neutrophil MMPs contributing to BBB breakdown.[7] On the other hand, monocytes expressing MMPs have been shown to be prominent contributors of BBB opening during multiple sclerosis.[8] These data support selective involvement of monocyte expressed MMPs in BBB disruption.

The mechanism of circulating monocyte activation in acute stroke is not well characterized and there are at least three possible explanations. First, preexisting vascular risk factors, including hypertension, may cause monocyte activation.[9] A second possible explanation is that the ischemic stroke itself may cause monocyte activation.[10] A recent study in mice reported that transient cerebral ischemia resulted in bone marrow hematopoetic stem cell activation with an increased output of inflammatory monocytes and neutrophils, and a decline in the number of lymphocyte progenitors.[10] We cannot completely rule out that the rise of monocytes is a result of a larger BBB disruption that is secondary to larger baseline perfusion deficit and infarct growth, as these variables also were associated with HARM (Table). The relatively small sample size of this pilot study does not allow us to perform a robust logistic regression analysis to rule out such a possibility. Lastly, there is the possibility, that pre-stroke systemic inflammation could mediate BBB disruption in humans, an idea that is both plausible and intriguing. Alternatively, it is possible that in patients who are prone to reperfuse, and hence to develop HARM, there is some unknown ischemic tissue signaling molecule that triggers monocytes to be released in the circulation during reperfusion of the ischemic tissue. We hope this pilot data will encourage further exploration to identify a causal relationship.

In this study, HARM was detected in 27% of untreated patients with acute ischemic stroke presenting within 12 hours of last known well. These results are comparable to our previously reported 25% rate when IV rt-PA treated patients were excluded,[1] but lower than 40% reported by Rozanski et al.[11] However, the Rozanski et al cohort was older (> 80 years) and included patients treated with IV rt-PA.[11] Contrary to our previous study,[1] in the current study, although there was a trend, reperfusion did not predict HARM. This finding is similar the Rozanski et al report which also failed to detect an association between reperfusion and HARM.[11] Exclusion of IV rt-PA treatment in the current study might be a possible explanation for not replicating the previously reported association between reperfusion and HARM. Since this study excluded patients treated with IV rt-PA, it is possible that some patients reperfused beyond 24 hours and that is the reason why no association was detected on the 24-hour scan. Alternatively, similar to PRES or neurosarcoidosis, postcontrast FLAIR enhancement may represent biomarker of BBB disruption which is not related to reperfusion.[12,13]

Although MRA was part of the stroke MRI protocol, no data on recanalization status was collected. We evaluated reperfusion, not recanalization, as some small vessel occlusions might not be identified on MRA.

As in the previous study, in the current study, HARM was evaluated on the 24-hour follow-up scan to allow for the maximum accumulation of enhancement.[1] Since HARM was detected on the 24-hour follow-up scan we cannot completely rule out preexisting BBB disruption. Increased BBB permeability on dynamic contrast–enhanced MRI in association with white matter hyperintensities was recently reported in small vessel disease patients of Binswanger's type supporting the relationship between BBB disruption and the development of white matter disease.[14]

O'Connell GC et al have recently shown that peripheral blood AKAP7 expression is an early marker for lymphocyte-mediated post-stroke HARM.[15] Since major source of AKAP are lymphocytes, it is difficult to correlate an increase in monocytes with AKAP and BBB disruption. However, as O'Connell et al suggest, it is possible that lymphocyte extravasation leads to lymphocyte-mediated production of pro-inflammatory chemokines which increases recruitment of innate immune cell populations such as neutrophils and monocytes from the periphery into the central nervous system.[15]

This study has some limitations. Relatively small sample size is one of the limitations of this pilot study and, as was already mentioned above, it did not allow to conduct robust logistic regression analysis. Our inclusion criteria, of 1) early presentation, 2) no reperfusion therapy, 3) consent to research, and 4) multiple imaging visits, greatly limited the sample size. Lack of information about stroke etiology is another limitation of the study as it is possible that different stroke subtypes may be associated with higher monocyte counts. In this study, reperfusion and DWI lesion growth were calculated volumetrically rather than with a voxel-based method which may also be a methodological shortcoming.

However, the findings of this retrospective pilot study have shifted our view in favor of systemic inflammation playing a causal role in HARM. This is intriguing and worth further exploration as it could provide a marker to target for treatment. We need to further study the interaction between inflammation and other factors predictive of HARM prospectively in a larger population.

Although sensitivity and specificity of HARM in detection of BBB disruption in stroke has not been tested, HARM has been shown to be caused by leakage of gadolinium-based contrast agents into the cerebrospinal fluid.[16] In addition, since gadolinium-containing MR contrast agents have a considerable molecular size, their transfer across the intact BBB is unlikely.[17] HARM is considered as a valid marker of BBB disruption. Recently, the frequency of post contrast FLAIR enhancement was reported at a much lower frequency in multiple sclerosis as compared with stroke.[18] It is possible that in early stages of MS, BBB disturbance in MS is focal and limited to the site of an inflammation while the remainder of the vascular bed is intact.[18]

Although the findings of the current study are valid, they cannot be generalized to the standard population of acute ischemic stroke patients presenting within 6 hours of onset and treated with reperfusion therapies as the current study excluded these patients.

In conclusion, in this pilot study, abnormally elevated monocyte count was associated with HARM, which suggests that monocytes may be playing a role in the development of BBB disruption after ischemic stroke.

## Acknowledgments

## References

1. Barr TL, Latour LL, Lee KY, et al. Blood-brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9. Stroke. 2010; 41:123–8.

2. Yang Y, Rosenberg GA. Matrix metalloproteinases as therapeutic targets for stroke. Brain research. 2015; 16:30–8.

3. Zhang Y, McCluskey K, Fujii K, et al. Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF-alpha, granulocyte-macrophage CSF, and IL-1 beta through prostaglandin-dependent and -independent mechanisms. J Immunol. 1998; 161:3071–6. [PubMed: 9743373]

4. Luby M, Bykowski JL, Schellinger PD, et al. Intra- and interrater reliability of ischemic lesion volume measurements on diffusion-weighted, mean transit time and fluid-attenuated inversion recovery MRI. Stroke. 2006; 37:2951–6. [PubMed: 17082470]

5. Urra X, Villamor N, Amaro S, et al. Monocyte subtypes predict clinical course and prognosis in human stroke. J Cereb Blood Flow Metab. 2009; 29:994–1002. [PubMed: 19293821]

6. Chiba T, Umegaki K. Pivotal roles of monocytes/macrophages in stroke. Mediators Inflamm. 2013; 75:1–10.

7. Maier CM, Hsieh L, Yu F, et al. Matrix metalloproteinase-9 and myeloperoxidase expression: quantitative analysis by antigen immunohistochemistry in a model of transient focal cerebral ischemia. Stroke. 2004; 35:1169–74. [PubMed: 15060315]

8. Bar-Or A, Nuttall RK, Duddy M, et al. Analyses of all matrix metalloproteinase members in leukocytes emphasize monocytes as major inflammatory mediators in multiple sclerosis. Brain. 2003; 126:2738–49. [PubMed: 14506071]

9. Dörffel Y, Lätsch C, Stuhlmüller B, et al. Preactivated peripheral blood monocytes in patients with essential hypertension. Hypertension. 1999; 34:113–7. [PubMed: 10406833]

10. Courties G, Herisson F, Sager HB, et al. Ischemic stroke activates hematopoietic bone marrow stem cells. Circ Res. 2015; 116:407–17. [PubMed: 25362208]

11. Rozanski M, Ebinger M, Schmidt WU, et al. Hyperintense acute reperfusion marker on FLAIR is not associated with early haemorrhagic transformation in the elderly. Eur Radiol. 2010; 20:2990–6. [PubMed: 20652257]

12. Weier K, Fluri F, Kos S, et al. Postcontrast FLAIR MRI demonstrates blood-brain barrier dysfunction in PRES. Neurology. 2009; 72:760–2. [PubMed: 19237706]

13. Wengert O, Rothenfusser-Korber E, Vollrath B, et al. Neurosarcoidosis: correlation of cerebrospinal fluid findings with diffuse leptomeningeal gadolinium enhancement on MRI and clinical disease activity. J Neurol Sci. 2013; 335:124–130. [PubMed: 24071064]

14. Huisa BN, Caprihan A, Thompson J, et al. Long-term blood-brain barrier permeability changes in Binswanger disease. Stroke. 2015; 46:2413–8. [PubMed: 26205374]

15. O'Connell GC, Treadway MB, Petrone AB, et al. Peripheral blood AKAP7 expression as an early marker for lymphocyte-mediated post-stroke blood brain barrier disruption. Sci Rep. 2017; 7:1172. [PubMed: 28446746]

16. Köhrmann M, Struffert T, Frenzel T, et al. The hyperintense acute reperfusion marker on fluid-attenuated inversion recovery magnetic resonance imaging is caused by gadolinium in the cerebrospinal fluid. Stroke. 2012; 43:259–61. [PubMed: 21980209]

17. Ewing JR, Knight RA, Nagaraja TN, et al. Patlak plots of Gd-DTPA MRI data yield blood– brain transfer constants concordant with those of 14C-sucrose in areas of blood–brain opening. Magn Reson Med. 2003; 50:283–92. [PubMed: 12876704]

18. Eisele P, Griebe M, Szabo K, et al. Investigation of leptomeningeal enhancement in MS: a postcontrast FLAIR MRI study. Neurology. 2015; 84:770–5. [PubMed: 25616480]

**Table**

Demographic, clinical, imaging and laboratory characteristics of patients

|  | All (n = 33) | HARM (+) (n = 9) | HARM (−) (n = 24) | p |
|---|---|---|---|---|
| Age (mean ± SD) | 72.9 ± 14.2 | 77.3 ± 15.1 | 71.2 ± 13.8 | 0.254 |
| Gender-Female %, (n) | 61 (20) | 44 (4) | 67 (16) | 0.235 |
| Onset to triage (min) (IQR**) | 168 (90–453) | 140 (78–238) | 185 (81–527) | 0.290 |
| Onset to baseline MRI (min) (IQR) | 224 (136–536) | 212 (136–326) | 249 (113–578) | 0.442 |
| Onset to 24-hour MRI (hours) (IQR) | 28 (26–33) | 27 (23–29) | 29 (27–34) | 0.118 |
| Hypertension %, (n) | 67 (22) | 78 (7) | 63 (15) | 0.421 |
| Diabetes %, (n) | 18 (6) | 22 (2) | 17 (4) | 0.745 |
| Admission NIHSS (IQR) | 8 (6–16) | 9 (8–17) | 8 (5–14) | 0.222 |
| Antiplatelet use %, (n) | 48 (16) | 44 (4) | 50 (12) | 0.762 |
| Statin use %, (n) | 33 (11) | 33 (3) | 33 (8) | 1.000 |
| Warfarin use %, (n) | 21 (7) | 22 (2) | 21 (5) | 0.951 |
| Baseline DWI volume [mL] (IQR) | 8.8 (3.9–21.6) | 5.1 (3.7–43.9) | 10.8 (3.6–19.4) | 0.858 |
| Baseline MTT volume [mL] (IQR) | 173.3 (67.2–217.1) | 219.4 (151.4–255.5) | 158.5 (32.7–198.6) | 0.029 |
| Baseline PD MM [mL] (IQR) | 138.5 (41.9–198.8) | 183.2 (115.0–215.9) | 129.8 (29.1–166.6) | 0.065 |
| DWI volume @ 24 hours [mL] (IQR) | 11.5 (4.7–43.2) | 23.7 (9.9–81.1) | 10.2 (2.9–35.6) | 0.142 |
| MTT volume @ 24 hours [mL] (IQR) | 86.7 (16.4–196.4) | 86.7 (36.9–181.6) | 76.6 (10.1–201.0) | 0.706 |
| FLAIR volume @ 5 days [mL] (IQR) | 14.2 (0.0–53.2) | 35.1 (8.5–54.8) | 10.2 (0.0–53.1) | 0.272 |
| DWI change @ 24 hours [mL] (IQR) | 3.62 (−0.1–21.9) | 18.5 (3.9–43.4) | 0.14 (−1.7–13.6) | 0.017 |
| Reperfusion volume @ 24 hours [mL] (IQR) | −26.8 (−107.5–8.0) | −104.6 (−150.0–1.9) | −16.6 (−66.3–9.5) | 0.135 |
| Infarct change @ 5 days [mL] (IQR) | 25.7 (4.1–49.3) | 30.2 (25.7–49.3) | 5.93 (0.83–49.2) | 0.142 |
| ESR [mm/hour] (IQR) | 12.0 (5.5–21.5) | 15.0 (4.5–35.5) | 11.5 (6.0–20.3) | 0.619 |
| WBC - $1 \times 10^3/mm^3$ (IQR) | 8.6 (7.2–9.2) | 8.7 (7.2–16.8) | 8.6 (6.2–9.1) | 0.254 |
| Neutrophils - $1 \times 10^3/mm^3$ (IQR) | 6.0 (4.8–7.5) | 6.0 (5.0–12.7) | 5.7 (3.8–7.3) | 0.328 |
| Lymphocytes-$1 \times 10^3/mm^3$ (IQR) | 1.5 (1.0–2.0) | 1.5 (1.3–2.3) | 1.5 (0.9–1.9) | 0.564 |
| Monocytes- $1 \times 10^3/mm^3$ (IQR) | 0.6 (0.5–0.8) | 0.9 (0.6–1.4) | 0.6 (0.4–0.7) | 0.011 |

Abbreviations: HARM -hyperintsense acute repefusion marker; m ± SD- mean ± standard deviation; IQR-interquartile range; NIHSS- National Institutes of Health stroke scale; min-minutes; DWI-diffusion weighted image; MTT- mean transit time; PD MM- perfusion diffusion mismatch; FLAIR- fluid attenuated inverse recovery; ESR-erythrocyte sedimentation rate; WBC -White blood cells.