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Metabolic Determinants of White Matter Hyperintensity Burden in Patients with Ischemic Stroke

Lisa Cloonan, BA^a, Kaitlin M. Fitzpatrick, BSc^a, Allison S. Kanakis, MD^a, Karen L. Furie, MD MPH^{a,1}, Jonathan Rosand, MD MSc^{a,b,c}, and Natalia S. Rost, MD MPH^{a,b}

^a J. Philip Kistler Stroke Research Center, Department of Neurology, Massachusetts General Hospital, Boston MA 02114

^b Center for Human Genetic Research, Massachusetts General Hospital, Boston MA 02114

^c Division of Neurocritical Care and Emergency Neurology, Department of Neurology, Massachusetts General Hospital, Boston MA 02114, USA

Abstract

Objective—Increasing white matter hyperintensity (WMH) burden is linked to risk of stroke and poor post-stroke outcomes. While the biology of WMH remains ill-defined, several lines of evidence implicate endothelial dysfunction. In this study, we sought to assess the association between metabolic markers of endothelial dysfunction and WMH severity in patients with acute ischemic stroke (AIS).

Methods—In this retrospective study, consecutive subjects, 18 years of age, admitted to our ED with AIS, brain MRI, and blood homocysteine (Hcy) and hemoglobin A1c (HgbA1c) measurements were eligible for this analysis. WMH volume (WMHV) was quantified using a validated semi-automated algorithm and log-transformed for linear regression analyses.

Results—: There were 809 AIS subjects included (mean age 65.57±14.7, median WMHV 6.25cm³ (IQR 2.8-13.1)). In univariate analysis, age, female gender, race, ethnicity, systolic blood pressure, history of hypertension, atrial fibrillation, coronary artery disease, prior stroke, and current alcohol and tobacco use (all $p < 0.05$), as well as Hcy ($p < 0.0001$) and HgbA1c levels ($p = 0.0005$) were associated with WMHV. However, only Hcy ($\beta = 0.11$, $p = 0.003$) and HgbA1c levels ($\beta = 0.1$, $p = 0.008$) independently predicted WMHV in the multivariate model, along with age ($\beta = 0.03$, $p < 0.0001$), race ($\beta = 0.39$, $p = 0.01$), ethnicity ($\beta = -0.11$, $p = 0.03$), and current alcohol use ($\beta = 0.26$, $p = 0.002$).

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Correspondence and Reprints: Natalia S. Rost, MD, MPH J. Philip Kistler Stroke Research Center Massachusetts General Hospital 175 Cambridge Street, Suite 300 Boston MA 02114 USA Tel: 001-617 643-3877 Fax: 001-617-726-3939 nrost@partners.org. lcloonan@partners.org, kaitlin.fitzpatrick@gmail.com, allison.kanakis@gmail.com, kfurie@lifespan.org, jrosand@partners.org

¹**Present Address** Department of Neurology, Rhode Island Hospital, Alpert Medical School of Brown University, 110 Lockwood St., Suite 324, Providence, RI 02903

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Conclusions—Elevated levels of Hcy and HgbA1c have been previously linked to endothelial dysfunction related to oxidative stress. The association between Hcy and HgbA1c and WMH burden in AIS suggests that the degree of endothelial dysfunction may be greater in patients with increased WMHV, and may in part explain the relationship between WMHV and poor post-stroke outcomes.

Keywords

White Matter Hyperintensity; hyperglycemia; hyperhomocysteinemia; endothelial dysfunction; acute ischemic stroke; blood biomarkers; small vessel disease

Introduction

White matter hyperintensity (WMH), also known as leukaraiosis, is an abnormality in cerebral tissue architecture, detected on neuroimaging and is most commonly present in the elderly and patients with stroke.^{1,2,3} WMH is considered to be a part of small vessel disease pathology; and therefore, WMH burden is greater in individuals with common cerebrovascular risk factors, such as hypertension, diabetes mellitus (DM), and smoking.^{1,4} The exact pathophysiology of WMH remains poorly understood, but some studies suggested that inflammatory markers, such as the intercellular adhesion molecule-1 (sICAM-1),⁵ and plasma markers linked to endothelial health such as homocysteine (Hcy) may contribute to WMH burden in addition to traditional vascular risk factors.⁶

The endothelium plays a key role in maintaining vascular tone, vessel-wall permeability, and thromboresistance.⁷ Atherogenic states including DM and hyperhomocysteinemia are thought to promote endothelial dysfunction via the metabolites associated with these conditions, such as plasma Hcy and chronic hyperglycemia (as measured by plasma levels of hemoglobin A1c (HgbA1c)), which increase oxidative stress, thrombogenicity, overactivation of redox-sensitive inflammatory pathways, and atherogenesis.^{8,9,10} In comparison to the previously validated experimental biomarkers of endothelial dysfunction,¹¹ such as sICAM-1, vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin and others, plasma Hcy and HgbA1c maintain relatively stable levels during acute events, are linked to the direct markers of endothelial function through common metabolic disease pathways, and importantly, are readily available for use in clinical practice.¹²⁻¹⁴ These characteristics render Hcy and HgbA1c as relevant and readily generalizable, albeit surrogate, biomarkers of endothelial function for use in applied clinical research.

In this study, we hypothesized that severity of WMH is linked, in part, to the degree of endothelial dysfunction, and this relationship can be assessed through the use of commonly available clinical biomarkers of endothelial function such as Hcy and HgbA1c. In this analysis, we examined the association between the plasma levels of Hcy and HgbA1c and the severity of WMH measured on the brain MRI of patients with ischemic stroke.

Methods

Subjects and data collection

All consecutive patients 18 years old, arriving to Massachusetts General Hospital Emergency Department (MGH ED) between July 2000 and October 2010, with signs and symptoms of acute ischemic stroke (AIS) were considered for this study. In this retrospective analysis of prospectively collected data, we included all consenting patients with: (1) evidence of acute cerebral infarct confirmed by diffusion-weighted imaging (DWI), (2) admission T2 fluid attenuated inversion recovery (FLAIR) MRI sequences available for volumetric WMH analysis, and (3) plasma Hcy and HgbA1c values drawn upon hospital admission. The Partners Institutional Review Board (IRB) approved of human subjects participation in this study. IRB-approved subject or proxy informed consent was obtained prior to subjects' enrollment into the study.

Data were obtained through medical record review and/or in-person interviews. Baseline clinical characteristics and laboratory values, including plasma Hcy and HgbA1c levels were obtained on ED admission. Based on MGH Pathology service classification, normal ranges of Hcy and HgbA1c values are defined as 0-12 $\mu\text{mol/L}$ and 3.8-6.4%, respectively.

Neuroimaging protocol and analysis

We have previously published a volumetric analysis method used to assess WMH severity in this cohort.¹⁵ The semi-automated protocol used MRI scans obtained from a 1.5T scanner. Scans acquired closest to stroke onset were analyzed. Prior to analysis, MRI scans were converted from DICOM into Analyze format through the use of MRICro (University of Nottingham School of Psychology, Nottingham, UK; www.mricro.com). DWI and T2FLAIR sequences were collated and cross-referenced to identify and exclude edema, hemorrhages, and infarcts from the WMHV measurement. WMHs present in supratentorial region were manually outlined and saved as a region of interest (ROI). A ROI matching the signal threshold of the WMH was created and intersected with the manually outlined WMH ROI. The intersection of these two ROIs was then manually touched up by a trained reader. The total WMHV was calculated by doubling the WMHV on the hemisphere unaffected by the stroke. Patients who presented with bilateral supratentorial lesions or brainstem infarcts were included in this analysis by combining both hemispheres' WMHVs to determine the total WMHV. To control for variation in head size, the intracranial area (ICA) was measured from two midline sagittal T1 slices, and then the total WMHV was multiplied by the individual- to-the mean ICA ratio.

Statistical analysis

WMHV values adjusted for head size were log-transformed for all analyses in this study. All variables were reported as either a mean value ($\pm$ standard deviation (SD)), a median value with an interquartile range (IQR), or a proportion/percentage of total. Univariate linear regression analysis was used to assess the association between WMHV and clinical variables, including quartiles of Hcy and HgbA1c. Those variables that reached a p-value <0.10 in univariate analysis were used in the multivariate linear regression analysis of WMHV. Statistical significance was set at p-value <0.05 in all analyses. Quantitative

variables were treated as continuous variables. Quartiles of plasma Hcy and HgbA1c levels were derived and used in univariate and multivariate linear regression analyses. The statistical analysis was conducted using the SAS 9.1 statistical packages (SAS Institute Inc, Cary NC).

Experimental Results

Demographic and clinical data are reported in **Table 1**. There were 809 subjects included in this analysis (mean age 65.57 ± 14.69 years, 37% female), of whom 750 (92.8%) were White and 718 (88.9%) were non-Hispanic. The median WMHV in this cohort was 6.25cm^3 (IQR 2.8-13.1).

In univariate analysis, Hcy ($\beta=0.23$, $p<0.0001$), HgbA1c ($\beta=0.12$, $p=0.0005$) (**Figure**), as well as age ($\beta=0.04$, $p<0.0001$), White race ($\beta=0.63$, $p<0.0001$), non-Hispanic ethnicity ($\beta=-0.19$, $p=0.0002$), female gender ($\beta=0.24$, $p=0.004$), admission systolic blood pressure ($\beta=0.008$, $p<0.0001$), prior history of hypertension ($\beta=0.55$, $p<0.0001$), atrial fibrillation ($\beta=0.49$, $p<0.0001$), coronary artery disease ($\beta=0.29$, $p=0.005$), current alcohol ($\beta=0.31$, $p=0.0001$) and tobacco use ($\beta=-0.32$, $p=0.002$), and prior stroke ($\beta=0.25$, $p=0.02$) were all associated with WMHV (**Table 2**). However, only Hcy ($\beta=0.11$, $p=0.003$), HgbA1c ($\beta=0.10$, $p=0.008$), as well as age ($\beta=0.03$, $p<0.0001$), White race ($\beta=0.39$, $p=0.01$), non-Hispanic ethnicity ($\beta=-0.11$, $p=0.03$), history of hypertension ($\beta=0.19$, $p=0.06$) and alcohol use ($\beta=0.26$, $p=0.002$) were independently associated with the greater burden of WMHV (**Table 3**). Each quartile increase in plasma Hcy was associated with WMHV increase of 11%; similarly, per-quartile increase in HgbA1c levels was associated with 10% in WMHV.

Discussion

Our results demonstrate that in patients with ischemic stroke, metabolic markers of endothelial dysfunction such as plasma Hcy level and chronic blood glucose levels, as measured by HgbA1c, are associated with the severity of WMH. These data provide novel, preliminary evidence of a link between the state of endothelial health and severity of WMH in patients with ischemic stroke, adding to the growing body of knowledge regarding the pathophysiology of WMH.

Endothelial dysfunction has long been linked to severity of WMH in a number of studies highlighting the role of increased blood-brain barrier (BBB) permeability,¹⁶⁻²⁰ which results from disruption of cell gap junctions and increased vascular permeability.⁷ Further, endothelial dysfunction manifests in a complex cascade of pathophysiological processes that culminate in microvascular failure and include endothelial up-regulation of adhesion molecules,⁷ vascular smooth muscle tone dysregulation due to reduced endothelial nitric oxide synthase activity (eNOS) production,⁷ and smooth muscle cell activation leading to cellular proliferation and migration.²¹ These dysregulated processes can be monitored in subjects with white matter disease and stroke using plasma biomarkers of endothelial health.²⁰

An important strength of our study is its clinical relevance through the use of commonly available biomarkers. While a number of experimental plasma biomarkers (such as

sICAM-1, VCAM-1, P-selectin, E-selectin, and others) have been associated with endothelial functional state, their utility has been significantly limited in applied clinical research. The novelty of our approach is in utilizing metabolic markers broadly linked to, and also frequently used in clinical surveillance of the endothelial dysfunction in ischemic stroke patients. Hyperhomocysteinemia can provoke endothelial dysfunction through oxidative stress, atherogenesis, increased thrombogenicity, and over-activation of the redox-sensitive inflammatory pathways.^{8,9,22} Furthermore, Hcy causes asymmetric dimethylarginine (ADMA) accumulation;²³ thus, inhibiting eNOS activity in vascular endothelium and reducing blood flow,²⁴ which has been linked to WMH severity in adults with cardiovascular disease.²⁵ Elevated circulating levels of ADMA, an endogenous inhibitor of NOS, is also considered an independent marker of endothelial injury and dysfunction.^{24,26} ADMA levels are shown to parallel stroke severity and may contribute to brain injury by attenuating cerebral blood flow, facilitating oxidative stress, and promoting inflammatory responses.²⁴

As in hyperhomocysteinemia, ADMA accumulation is observed in diabetics and those with insulin resistance.²⁴ DM or insulin resistance state, represented by the surrogate marker HgbA1c, can trigger oxidative stress, increasing the synthesis and decreasing degradation of ADMA.²⁴ ADMA also promotes the development of atherosclerosis in the carotid arteries,²⁷ which parallels the finding of a greater degree of carotid artery atherosclerosis found in individuals with elevated levels of HgbA1c.²⁸

Whether a direct link in the pathophysiological cascade (like Hcy), or an indicator of chronically elevated blood glucose levels associated with DM or insulin resistance (like HgbA1c), these plasma metabolites mark the state of endothelial dysfunction and predict severity of WMH in patients with ischemic stroke. Both hyperhomocysteinemia and DM can result in endothelial dysfunction through ADMA accumulation, which is linked to a reduction in cerebral perfusion and accelerated atherosclerosis, potentially contributing to WMH burden. Age, another independent predictor of WMH severity that emerges consistently in our study,^{6,15,29} may have similar effects on small cerebral vasculature,³⁰ including its role in BBB permeability.¹⁷

The magnitude of alcohol consumption has been linked to increasing WMH burden.^{31,32} In this large cohort of ischemic stroke patients, current alcohol consumption independently predicted greater WMHV. Alcohol has a known effect on endothelium, which can be either protective^{33,34} or detrimental.^{35,36} However, alcohol consumption can trigger neuroinflammatory cascade, releasing NF- κ B, caspase-3 and pro-inflammatory cytokines (TNF- α and IL-1 β)³⁷ and chronic alcohol consumption can induce oxidative stress, such as oxidative-nitrosative stress, which contributes to cognitive dysfunction.^{38,39} Based on our findings, we posit that alcohol consumption may contribute to endothelial dysfunction in subjects with multiple vascular risk factors, and thus, to severity of WMH detected on brain MRI at the time of AIS.

Our data on the effects of race and ethnicity on WMH burden are not aligned with the previous reports that demonstrated correlation between the minority status and severity of WMH.⁴⁰⁻⁴² Considering that one of the major limitations of our study is lack of racial and

ethnic diversity among our patient population (93% Caucasian race, 89% non-Hispanic ethnicity), we may be at a disadvantage to fully explore the effects of population stratification on WMH severity. Another notable discrepancy in our findings is that the association between hypertension status and WMHV has not reached the statistical significance threshold in multivariable analysis. Hypertension is a validated risk factor for WMH, which has been particularly well-defined within the population-based studies of white matter disease.¹⁻⁴ The majority of AIS subjects in our cohort were hypertensive (63.4%), which could have limited the statistical model's ability to differentiate the independent effect of hypertension on WMHV, despite an apparent trend ($\beta=0.19$, $p=0.06$). A larger sample size to replicate our findings would be needed to ensure adequate statistical power to detect the effect of hypertension on WMH in a hospital-based sample of patients with ischemic stroke.

Other limitations of this study are largely attributed to its retrospective design. Plasma biomarkers examined in this analysis have been measured as part of clinical investigations, and while the timeline of blood sampling ranged between the subjects, it did not exceed the maximum of 5 days from stroke onset. This limitation may have the greatest effect on the measured Hcy levels, given that plasma Hcy half-time is about 6.5 hours; however, the overall distribution of plasma Hcy in our study (median value of 8.90 $\mu\text{mol/L}$, IQR 7.2-11.4) mimics closely previously reported values in stroke patients.^{43,44} The study's reliance on clinically obtained plasma biomarkers is a technical limitation forcing the use of surrogate metabolites of endothelial dysfunction as opposed to experimentally validated, direct biomarkers such as VCAM-1 and ICAM-1; however, using clinical data offers a pragmatic and generalizable approach, provided that our findings can be validated in the future studies. Furthermore, testing of other clinically relevant markers of endothelial dysfunction (such as proteinuria) in a number of vascular⁴⁵ and metabolic disorders⁴⁶ may improve our current understanding of the underlying disease biology. The main strengths of this study are its relatively large size; systematic collection of the clinical, neuroimaging, and laboratory characteristics in all consecutive patients with ischemic stroke; and in particular, the quantitative approach to WMH assessment using a validated, volumetric method that ensures specific representation of the existing white matter disease.

In summary, this analysis further explored the underlying mechanisms of white matter disease by uncovering the association between plasma metabolites linked to endothelial dysfunction and WMHV in a large cohort of patients with ischemic stroke. Future studies are needed to validate these findings and to further explore the biology linking the dysregulated endothelium and chronic cerebrovascular disease.

Conclusions

Endothelial dysfunction is the major risk factor for atherosclerosis. The link between WMH severity and the markers of endothelial dysfunction, as demonstrated in our analysis, may provide further insight into the underlying biology of cerebrovascular disease, including the role of atherosclerosis. Use of clinically relevant biomarkers in this study of a large hospital-based cohort of patients with ischemic stroke provides future opportunities for cross-validation of the data and hypothesis-driven investigations in WMH research.

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Highlights

- White matter (WM) disease may be linked to endothelial health in stroke patients
- We examined a large hospital-based stroke cohort with MRI & serum metabolic markers
- We assessed WM disease burden using a validated volumetric MRI analysis method
- Elevated homocysteine and HgbA1c were independent predictors of WM disease
- Our data suggest that endothelial dysfunction may contribute to WM disease severity

Figure. White matter disease severity and plasma biomarkers of endothelial dysfunction

White matter hyperintensity volume measured on brain T2 FLAIR MRI of patients with ischemic stroke is significantly associated with plasma levels of homocysteine (*panel A*) and hemoglobin A1c (*panel B*). Y-axis: natural log-transformed white matter hyperintensity volume; X-axis: natural log-transformed homocysteine (*panel A*), natural log-transformed hemoglobin A1c (*panel B*).

Abbreviations: ln(WMHV), natural log-transformed white matter hyperintensity volume; ln(HgbA1c), natural log-transformed hemoglobin A1c

Table 1

Demographic and clinical characteristics of 809 subjects with ischemic stroke

Variables	
	<i>Mean (SD)</i>
Age (<i>years</i>)	65.57 (14.7)
Systolic Blood Pressure (<i>mmHg</i>)	151.76 (29.5)
Diastolic Blood Pressure (<i>mmHg</i>)	79.62 (15.4)
lnWMHV	1.83 (1.14)
	<i>Median (IQR)</i>
nWMHV (<i>cm³</i>)	6.25 (2.8-13.1)
Homocysteine (<i>μmol/L</i>)	8.90 (7.2-11.4)
Hemoglobin A1c (%)	5.90 (5.5-6.5)
Creatinine (<i>mg/dl</i>)	1.00 (0.8-1.2)
NIHSS score	3.00 (1-7)
	<i>N (%)</i>
Gender (<i>female</i>)	299 (37)
Race (<i>White</i>)	750 (92.8)
Ethnicity (<i>non-Hispanic</i>)	718 (88.9)
Hypertension	507(63.4)
Diabetes mellitus	165 (20.4)
Atrial fibrillation	118(14.6)
Coronary artery disease	155 (19.2)
Hyperlipidemia	326 (40.3)
Prior TIA	64 (7.9)
Prior Stroke	126 (15.6)
Tobacco Use	159 (20.1%)
Alcohol Use	420 (53.2%)

Abbreviations: β, beta; SD, standard deviation; lnWMHV natural log transformed white matter hyperintensity volume; IRQ, interquartile range; nWMHV, normalized white matter hyperintensity volume; NIHSS, National Institutes of Health Stroke Scale; TIA, transient ischemic attack.

Table 2

Univariate determinants of white matter hyperintensity volume in patients with ischemic stroke

Variables	β	p value
Homocysteine * ($\mu\text{mol/L}$)	0.23	<0.0001 [†]
Hemoglobin A1c * (%)	0.12	0.0005 [†]
Age (years)	0.04	<0.0001 [†]
Gender (female)	0.24	0.004 [†]
Ethnicity (non-Hispanic)	-0.19	0.0002 [†]
Race (White)	0.63	<0.0001 [†]
Systolic blood pressure (mmHg)	0.008	<0.0001 [†]
Diastolic blood pressure (mmHg)	0.004	0.11
Creatinine (mg/dl)	0.02	0.85
Hypertension	0.55	<0.0001 [†]
Diabetes mellitus	0.16	0.11
Atrial fibrillation	0.49	<0.0001 [†]
Coronary artery disease	0.29	0.005 [†]
Hyperlipidemia	0.07	0.39
Prior TIA	-0.09	0.56
Prior Stroke	0.25	0.02
Tobacco use	-0.32	0.002 [†]
Alcohol use	0.31	0.0001 [†]

Abbreviations: WMHV, white matter hyperintensity volume; β , beta.

* quartiles

[†] statistically significant

Table 3

Independent predictors of natural log-transformed WMHV in patients with ischemic stroke

Variable	β	p value
Homocysteine * ($\mu\text{mol/L}$)	0.11	0.003 [†]
Hemoglobin A1c * (%)	0.10	0.008 [†]
Age (years)	0.03	<0.0001 [†]
Gender (female)	0.13	0.13
Race (White)	0.39	0.01 [†]
Ethnicity (non-Hispanic)	-0.11	0.03 [†]
Hypertension	0.19	0.06
Diabetes	-0.15	0.26
Atrial Fibrillation	0.08	0.56
Coronary Artery Disease	-0.03	0.76
Prior Stroke	0.16	0.17
Tobacco Use	0.01	0.91
Alcohol Use	0.26	0.002 [†]

Abbreviations: WMHV, white matter hyperintensity; β , beta.

* quartiles

[†]=statistically significant