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Hemostatic markers and long-term risk of intracerebral hemorrhage in postmenopausal women

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Abstract

Background—Known risk factors for intracerebral hemorrhage (ICH) include age, hypertension, smoking, alcohol intake, and anticoagulant use. Some previous reports have indicated that hemostatic factors measured many years before the onset of ICH might predict the later occurrence of ICH. The objective of this analysis was to test whether selected hemostatic factors measured years before the onset of ICH could identify patients at higher risk for future ICH.

Methods—We performed a nested case-control study within the Women's Health Initiative (WHI) cohort. Postmenopausal women aged 50 to 79 years (mean 68) at baseline (1993-1998) were enrolled at 40 Clinical Centers in the United States and followed for adjudicated ICH for a mean of 11.4 years. ICH cases (N=75) and controls (N=75) were matched on age, ethnicity, blood pressure, anticoagulant use, and treated hypertension. Stored blood samples from the baseline WHI examination were tested for Von Willebrand Factor (VWF), ADAMTS13, tissue plasminogen activator (t-PA), and urokinase plasminogen activator (u-PA). Platelet count, white blood cell count, and hemoglobin concentration were also measured.

Results—Mean baseline levels of VWF (1.03 and 0.95 U/ml), ADAMTS13 (1.0 and 1.1 μg/ml), VWF:ADAMTS13 (0.99 and 0.92), t-PA (14.75 and 14.80 IU/mL), and u-PA (0.09 and 0.10 IU/mL) were not significantly different by case-control status. Significant differences were also not identified for platelet count, hemoglobin, white blood count, or reported alcohol use.

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Roles of Authors: J.M. Lee wrote the first draft. P. Greenland revised the intellectual content of manuscript and supervised the study. J.M. Lee and J. Siddique analyzed and interpreted data. D. Green measured the hemostasis biomarkers, analyzed and interpreted the data. H.C. Kim, L. V. Horn, M. Allison, S. Wassertheil-Smoller developed study concept and design. All authors approved of the final version.

Disclosures: None

Conclusion—None of the 4 baseline hemostatic factors, nor the platelet count, were predictive of future ICH risk in this long-term study of older postmenopausal women.

Keywords

Cerebral hemorrhage; Von Willebrand factor; ADAMTS13; Plasminogen Activators

Introduction

Intracerebral hemorrhage (ICH) is a common and frequently devastating disorder that accounts for up to 20% of all strokes [1,2]. In contrast to ischemic stroke that is often preceded by transient ischemic attack (TIA), ICH typically has no identifiable premonitory symptoms. Notably, mortality and morbidity of ICH are high (up to 50% at 30 days). Therefore, efforts to identify high-risk individuals could be especially important and useful in developing early preventive approaches [1-3]. In this regard, previous studies have identified older age, higher alcohol intake, hypertension, male sex, current smoking, and diabetes as risk factors [4] for the development of ICH. Lower blood cholesterol and lower triglycerides have also been reported in association with higher risk of primary ICH [5].

Several prior studies have suggested that hemostatic factors might also be related to subsequent occurrence of ICH. Consistent associations have been reported with ICH and use of anticoagulant agents [3]. Both warfarin [1,2,5] and aspirin [1,2,6] have been associated with an increased risk of ICH. Patients with inherited or acquired bleeding disorders also have a higher risk of ICH compared to those without bleeding disorders [3,7-11]. In long-term cohort studies, Von Willebrand Factor (VWF) has been examined in 2 studies, but results were inconsistent [10,12]. In one study, VWF was associated with higher risk of ICH [10], but in the second study, higher levels of VWF were significantly associated with lower ICH risk [12]. ADAMTS13 (a disintegrin-like and metalloprotease domain with thrombospondin type-1 motif, number 13) cleaves VWF high molecular weight multimers, and has been implicated in bleeding associated with the acquired Von Willebrand Syndrome [13]. Either increases in ADAMTS13 or a decrease in the ratio of VWF to ADAMTS13 could predispose to hemorrhage. The administration of tissue plasminogen activator (t-PA) or urokinase plasminogen activator (u-PA) for the treatment of acute pulmonary embolism is associated with an increased risk for intracranial hemorrhage [14] and higher levels of u-PA promote bleeding in patients with the Quebec platelet disorder [15]. Based on these previous studies, we hypothesized that higher levels of one or more of these hemostatic factors present years before an event might be predictive of subsequent ICH on long-term follow-up.

Materials and methods

1) Study population

The Women's Health Initiative (WHI) observational study (OS) was designed to investigate strategies for prevention of common diseases among postmenopausal women, including cancers, cardiovascular diseases, and osteoporotic fractures [6]. Detailed methods for the WHI study have been reported [6]. Postmenopausal women aged 50 to 79 years at baseline

(1994-1998) were enrolled at 40 Clinical Centers in the United States [16]. Overall 93,676 women participated in the observational study and were followed up for a mean of 16 years. For this analysis average follow up time was 11.4 years (range 4 month to 18 years). Participants provided written informed consent [6]. The study was approved by the institutional review boards of the participating clinical centers, the WHI coordinating center at the Fred Hutchinson Cancer Center, and the National Institutes of Health[16].

2) Design

Overall—This was a nested case-control study utilizing WHI baseline data, detailed event follow-up, and stored baseline blood specimens. Repeat blood sampling during follow-up was not available.

Matching—Cases of ICH (N=75) and controls without ICH (N=75) were matched by the WHI Coordinating Center on baseline factors for age (within 2 years), ethnicity (black/white), systolic BP (within 10 mm Hg), diastolic BP (within 10 mm Hg), aspirin use (≥ 80mg/day for 30 days as positive, yes/no), anticoagulant use (yes/no), or miscellaneous hematological drug use (anti-hemophilic products, platelet aggregation inhibitors, plasma expanders, plasma proteins, protamine, thrombolytic enzymes, yes/no), treated hypertension (yes/no), randomization clinic, blood draw dates (within 180 days), and follow up time (control was required to have at least as much follow up time as its matched case).

Exclusion criteria—1) not White or African American, 2) history of CVD (MI, CABG, PTCA, Stroke), 3) missing baseline variables or event follow up data, 4) inadequate stored baseline citrated plasma, 5) stroke (controls).

Cases and controls—All cases of intracerebral hemorrhagic (ICH) stroke were eligible to be selected, defined in WHI as a stroke classified as “intra parenchymal hemorrhage” based on central adjudication after local event adjudication [17]. Matched controls were eligible among those who did not have an ICH. Per WHI protocol [17], stroke was defined as: 1) Rapid onset of persistent neurological deficit attributable to obstruction or rupture of vascular system; 2) Deficit not known to be secondary to brain trauma, tumor, infection, or other cause; 3) Deficit lasting more than 24 hours, unless death supervenes or there is a demonstrable lesion compatible with acute stroke on CT or MRI scan. Stroke was further classified into 5 categories: 1) Subarachnoid hemorrhage not resulting from a procedure; 2) ICH not resulting from a procedure; 3) other or unspecified intracranial hemorrhage not resulting from a procedure; 4) occlusion of cerebral or precerebral arteries with infarction not resulting from a procedure; 5) and central nervous system complications during or resulting from a procedure. For this analysis, only ICH was included. With 75 cases and 75 controls, we have 80% power to detect a difference as small as 0.15 U/ml for VWF, 0.068 μg/ml for ADAMTS13, 10.00 IU/ml for t-PA, and 0.017 IU/ml for u-PA.

3) Biomarkers

VWF, ADAMTS13, VWF: ADAMTS13 ratio, t-PA, and u-PA.

Participant plasma samples that had been collected in sodium citrate, centrifuged, and stored frozen, were shipped from the WHI Coordinating Center to the Northwestern University Special Coagulation Laboratory, where they were thawed and assayed. For quality control, eight samples were split and assayed by a technician blinded to their identity. VWF antigen was measured using an enzyme-linked immunosorbent assay (ELISA, REAADS Von Willebrand Factor Antigen Test Kit, Corgenic, Inc., Broomfield, Colorado). The coefficient of variation (CV) for this assay was 10.1%. ADAMTS13 was assayed using an ELISA method (TECHNOZYM ADAMTS13 Antigen ELISA kit, DiaPharma, West Chester, Ohio). The CV of this assay was 7.4%. For the ratio calculation, VWF (U/ml) was divided by ADAMTS13 (μg/ml). Tissue plasminogen activator (t-PA, IU/ml) was measured using an ELISA kit (Assaypro LLC, St Charles, Missouri). The CV was 8.6%. Urokinase plasminogen activator (u-PA, IU/ml) was quantitated using a chromogenic activity assay (u-PA Human Chromogenic Activity Assay kit, Abcam, Inc., Cambridge, MA). The CV was 17.8%.

4) Statistical analysis

Differences between cases and matched controls were analyzed by paired T-test, and chi-square test. Analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA). A p-value <0.05 was considered to be statistically significant using a two-tailed test.

Results

Characteristics of the 75 cases and matched 75 controls are shown in Table 1. By design, there were no differences between groups for age, sex (all women), race, systolic BP, diastolic BP, aspirin/ anticoagulant use, and treated hypertension. While we did not match on additional parameters, there were also no significant differences in levels of hematocrit or hemoglobin, white blood count, platelet count, or alcohol drinking history. In addition, the mean concentrations of the biomarkers of interest (VWF, ADAMTS13, t-PA, u-PA and VWF:ADAMTS13 ratio) were not significantly different between cases and matched controls (Table 2).

Discussion

This study of ICH conducted in post-menopausal women, followed long-term for development of a host of disease endpoints including intracerebral hemorrhage, found no significant differences between cases and matched controls for VWF, ADAMTS13, vWF:ADAMTS13 ratio, t-PA, u-PA, and the platelet count. There were also no significant differences in other potential ICH risk factors including white blood count, hemoglobin, or hematocrit. Therefore, the study provided no support for the concept that specific hemostatic factors measured long before the onset of ICH could be useful in identifying a high-risk subgroup for early preventive efforts.

This study must be considered in the context of previous long-term follow-up studies for risk of ICH. A small number of previous studies of hemostatic factors (VWF, ADAMTS13, t-PA) and long-term risk of hemorrhagic stroke has been reported. One nested case referent study [12] from Sweden, with only 39 ICH cases, showed highest tertile of VWF versus lowest

tertile was protective for ICH (OR, 0.27; 95% CI, 0.08 to 0.90). However, in a larger cohort study involving two separate long-term cohorts [10] from the United States, including 135 total cases of ICH, a non-significant adverse effect was found for VWF above the median compared with below the median (RR, 1.72; 95% CI, 0.97 to 3.03). In the latter study, which included more than 260,000 person-years of follow-up, with median follow-up time of 13.5 years for participants free of ICH, and median time to event of 8.0 years for participants experiencing an ICH, higher baseline level of fibrinogen was associated with a relative rate of incident ICH, significantly increased by 35%. In this pooled analysis, Factor VIII was significantly positively related to ICH in one of the 2 studies (relative rate per standard deviation of 1.31; 95% CI, 1.07 to 1.62), but not in the second study. There was no relation in multivariable models between lipoprotein (a), Factor VII, white blood cell count, or C-reactive protein and ICH in this pooled cohort analysis. In murine studies, administration of ADAMTS13 ameliorated subarachnoid hemorrhage by reducing cerebral microvascular thrombosis and brain injury [18,19]. A nested case control study [20] failed to show that t-PA (OR, 0.63; 95% CI, 0.32 to 1.22) was associated with hemorrhagic stroke in 83 ICH cases. The Swedish nested case referent study [12] also failed to show that t-PA (OR, 1.07; 95% CI, 0.40 to 2.86) was associated with ICH (n=39).

In the previously discussed pooled cohort study [12], it was suggested that there is a possible interaction between hypertension and VWF for risk of ICH. Since we matched on hypertension at baseline, we could not address the question of synergy or interaction between any of the matched factors (e.g., age, hypertension, anticoagulant use) and the blood factors.

Limitations of this study should be acknowledged. One important limitation of this study is the relatively small number of ICH cases. It is possible that small numbers of ICH cases may have produced the null results here. However, based on power calculations, only very small differences may have been missed. From a clinical perspective, the small differences below our detection limit would not be useful for discriminating low risk people from high risk people. Also this study was larger than several previous studies with ICH cases [12,21] and we found no differences that were close to statistically significant. Another important limitation is that our design precluded repeated measurements of the hemostatic factors over time. It is possible that hemostatic factors measured closer to the time of ICH may be associated with ICH. However our study had only a one time measurement from baseline stored blood, therefore the current study design cannot rule out this possibility. Despite its limitations, the null findings here are important since they indicate that these factors measured many years before onset of ICH are not likely to be useful in identifying a high-risk group for early preventive interventions. Such interventions should focus, instead, on well-proven long-term risk factors such as hypertension, cigarette smoking, and diabetes.

In conclusion, we found no evidence that levels of specific hemostatic biomarkers present many years before the onset of ICH are risk factors for subsequent ICH. We therefore found no support for a larger-scale prospective cohort study of these markers for long-term risk of primary ICH in older postmenopausal women and no support for measuring these markers as risk factors for ICH.

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For a list of all the investigators who have contributed to WHI science, please visit: https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

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Table 1
Participant's characteristics after matching

	Case (N=75)	Control (N=75)	p-value
Matched			
Age, y	68.0 ± 6.5	67.9 ± 6.6	N/A
Race, white %	71	71	N/A
Systolic BP, mmHg	131.5 ± 18.1	130.4 ± 17.7	N/A
Diastolic BP, mmHg	73.6 ± 7.8	74.3 ± 7.4	N/A
Aspirin/anticoagulant use, yes	28 (37.3%)	28 (37.3%)	N/A
Treated hypertension, yes	19 (25.3%)	19 (25.3%)	N/A
Unmatched			
Platelet, Kcell/microliter	238.0 ± 55.8 (N=73)	242.0 ± 58.3 (N=74)	0.668
Hematocrit, %	39.7 ± 3.0 (N=73)	40.1 ± 3.0 (N=74)	0.439
Hemoglobin, gm/dl	13.4 ± 1.0 (N=73)	13.5 ± 1.0 (N=74)	0.254
WBC, Kcell/microliter	5.8 ± 1.7 (N=73)	5.8 ± 1.4 (N=74)	0.967
Smoking status	(N=74)	(N=73)	0.377
Never Smoked	35 (47.3%)	41 (56.2%)	
Past Smoker	31 (41.9%)	28 (38.4%)	
Current Smoker	8 (10.8%)	4 (5.5%)	
Drinking status	(N=75)	(N=75)	0.614
Non drinker	7 (9.3%)	5 (6.7%)	
Past drinker	12 (16.0 %)	9 (12.0%)	
Current drinker	56 (74.7%)	61 (81.3%)	

Data are expressed as mean ± SD, or number (%).

Table 2
Levels of hemostatic biomarker in case and control groups

	Cases (N=75)	Control (N=75)	p-value
vWF, U/ml	1.0 ± 0.3 (N=71)	1.0 ± 0.4 (N=71)	0.97
ADAMTS13, μg/ml	1.0 ± 0.2	1.1 ± 0.1	0.10
t-PA, IU/ml	24.3 ± 28.2 (N=72)	20.1 ± 19.4 (N=75)	0.16
u-PA, IU/ml	0.1 ± 0.1	0.1 ± 0.1	0.77
vWF/ADAMTS13 ratio	1.1 ± 0.4 (N=71)	1.0 ± 0.4 (N=71)	0.38

Data are expressed as mean ± SD.

*P-value was accessed by paired t-test.