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Hemostasis biomarkers and incident cognitive impairment: the REGARDS study

SR Gillett[*], **LA McClure**[†], **PW Callas**[*], **EL Thacker**[‡], **FW Unverzagt**[§], **VG Wadley**[¶], **AJ Letter**[**], and **M Cushman**[*]

[*]Department of Medicine, Larner College of Medicine at the University of Vermont

[†]Department of Epidemiology and Biostatistics, Drexel University

[‡]Department of Health Science, Brigham Young University

[§]Department of Psychiatry, Indiana University School of Medicine

[¶]Department of Medicine, University of Alabama at Birmingham

[**]Department of Biostatistics, University of Alabama at Birmingham

Abstract

Background—Vascular risk factors are associated with cognitive impairment, a condition with substantial public health burden. We hypothesized that hemostasis biomarkers related to vascular disease would be associated with risk of incident cognitive impairment.

Methods—We performed a nested case control study including 1,082 participants with 3.5 years of follow-up in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a longitudinal cohort study of 30,239 black and white Americans ≥45 years old. Participants were free of stroke or cognitive impairment at baseline. Baseline D-dimer, fibrinogen, factor VIII, and protein C were measured in 495 cases who developed cognitive impairment during follow-up (based on abnormal scores on ≥2 of 3 cognitive tests) and 587 controls.

Results—Unadjusted ORs for incident cognitive impairment were 1.32 (95% CI 1.02, 1.70) for D-dimer >0.50 μg/mL, 1.83 (CI 1.24, 2.71) for fibrinogen >90th percentile, 1.63 (CI 1.11, 2.38) for factor VIII >90th percentile and 1.10 (CI 0.73, 1.65) for protein C < 10th percentile. There were no

Correspondence: Mary Cushman, MD, MSc. Larner College of Medicine at the University of Vermont, 360 South Park Drive, Colchester, VT 05446. Phone: 802-656-8968; mary.cushman@uvm.edu.
DR MARY CUSHMAN (Orcid ID: 0000-0002-7871-6143)

Addendum

S. Gillett participated in the study concept and design, analysis and interpretation of data, drafting the manuscript and its final approval. L. McClure participated in the study concept and design, analysis and interpretation of data, critical revision of the manuscript and its final approval. P. Callas participated in the study design, analysis and interpretation of data, critical revision of the manuscript and its final approval. E. Thacker participated in the study design, interpretation of data, critical revision of the manuscript and its final approval. F. Unverzagt participated in the study concept and design, interpretation of data, critical revision of the manuscript and its final approval. V. Wadley participated in the study concept and design, interpretation of data, critical revision of the manuscript and its final approval. A. Letter participated in the analysis and interpretation of data, critical revision of the manuscript and its final approval. M. Cushman participated in the study concept and design, analysis and interpretation of data, critical revision of the manuscript and its final approval.

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The authors state that they have no conflict of interest.

differences in associations by race or region. Adjustment for demographic, vascular and health behavior risk factors attenuated these associations. However, having at least 2 elevated biomarkers was associated with incident cognitive impairment, with an adjusted OR 1.73 (CI 1.10, 2.69).

Conclusion—Elevated D-dimer, fibrinogen, and factor VIII were not associated with occurrence of cognitive impairment after multivariable adjustment; however, having at least 2 abnormal biomarkers was associated, suggesting the burden of these biomarkers is relevant.

Keywords

cognitive impairment; hemostasis; thrombosis; risk factor; epidemiology

Dementia and cognitive dysfunction are prevalent, devastating and costly conditions[1] that are increasing as the population ages.[2] Although many risk factors for dementia have been identified in recent decades,[1] the underlying mechanisms for many of these associations are unknown. Biological processes that lead to dementia probably begin long before clinical signs of cognitive dysfunction are apparent. Identifying biomarkers of brain health may help detect disease at an earlier stage and identify targets that can be modified to slow or reverse disease progression.

Contributions of vascular disease and its risk factors to cognitive impairment and dementia are increasingly evident. [3] Since they are also vascular risk markers, biomarkers associated with hypercoagulable states might also relate to cognitive impairment. Hemostasis biomarkers that have been investigated in this regard include fibrinogen, D-dimer, factor VII, factor VIII, prothrombin fragment 1.2, von Willebrand factor (vWF), and plasminogen activator inhibitor-1 (PAI-1). There are few prospective studies of the relationship between these biomarkers and cognitive impairment or dementia, and they report inconsistent associations. The most extensively studied biomarker is fibrinogen. Six studies reported a direct association of higher fibrinogen with incident cognitive impairment Alzheimer Disease (AD), vascular dementia (VaD) or cognitive decline [4–9], and one study found no association with dementia [10]. One study reported an association of higher D-dimer with VaD but not AD [10], two reported an association with decline in cognitive test scores [6, 11] and two more reported no association with cognitive decline or dementia [4, 5]. Factor VIII has only been studied in one report and was associated with VaD but not other dementia or cognitive impairment [4]. A meta-analysis of D-dimer, fibrinogen, factor VII, prothrombin fragment 1.2, vWF and PAI-1 in relation to cognitive decline and dementia [12] reported small but potentially important associations, especially between D-dimer and VaD. Little research has been directed at the anticoagulant proteins. Low protein C was associated with stroke risk in one study [13] but we are not aware of studies of protein C and cognitive function. Racial differences in hemostasis biomarker levels have been identified, with blacks having a more procoagulant profile than whites [14, 15]. Only one study of hemostasis markers and risk of cognitive decline included large number of African Americans[11].

There are regional differences in incident cognitive impairment in the United States [16]; regions with higher risk of cognitive impairment overlap with the “stroke belt,” 8 southeastern United States (AL, NC, SC, GA, TN, MI, LA) with increased risk of stroke

incidence and mortality [17]. Whether abnormal levels of hemostasis biomarkers might mediate the geographic difference in incident cognitive impairment is unknown.

We examined the association of D-dimer, fibrinogen, factor VIII, and protein C with incident cognitive impairment (ICI) in a large national cohort of black and white Americans. We investigated whether associations differed by race and whether differences in these biomarker levels mediated any of the higher risk of ICI in the stroke belt. We previously reported that higher factor VIII was associated with ICI in this study in the context of ABO blood group, finding a minimally-adjusted odds ratio of 1.24 (95% CI 1.10, 1.38) per SD higher factor VIII [18], and here extend that analysis with more detail and inclusion of other biomarkers.

Methods

Subjects

The REGARDS study has been described in detail elsewhere[19]. In brief, the study enrolled 30,239 Americans age 45 and older from 2003–2007. Participants are 58% white and 42% black, 45% male and 55% female, 56% from the stroke belt region and 44% from the other 40 contiguous United States. Participants were recruited by random selection from a commercially available nationwide list. Exclusion criteria were race other than black or white, Hispanic ethnicity, active treatment for cancer, medical conditions that would prevent long-term participation, cognitive impairment judged by the telephone interviewer, residence in or inclusion on a waiting list for a nursing home, or inability to communicate in English. Following verbal consent, medical history including cardiovascular risk factors was collected by computer-assisted telephone interview. Thereafter, subjects participated in an in-home examination which included height, weight and blood pressure measurements, a resting ECG, medication inventory, and fasting blood and urine samples. Methods were approved by the institutional review boards of all participating institutions, and all participants provided written informed consent.

REGARDS includes a two-level longitudinal telephone-based assessment of cognitive function: 1) the Six-Item Screener[20] (SIS), added to the baseline assessment in 2003 and then performed annually, and 2) a 3-test cognitive battery introduced in 2006 and administered at 2-year intervals. This battery includes the Animal Fluency Test (AF),[21] Word List Learning (WLL) and Word List Recall (WLR) from the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) battery.[22] The SIS is a brief screening tool with 3 immediate recall items and 3 temporal orientation items. A score ≤4 represents cognitive impairment.[16, 23] The AF is a measure of verbal fluency in which the participant names as many animals as possible in 60 seconds. WLL and WLR consist of a 10-item, three-trial word list learning and recall task. The WLL score is the sum of immediate recall across the 3 trials, range 0–30. After the last learning trial the participant is asked non-cognitive interview questions for five minutes, and is then asked to provide a free recall of the word list resulting in the WLR score, range 0–10.

Covariates collected included age, race (black or white), sex, education (<high school, high school, some college, or college and above), yearly income (<$20,000, $20,000–34,999,

$35,000–74,999, ≥$75,000 or refused), region (stroke belt or non-stroke belt), cigarette smoking (never, past, or current), alcohol intake (none, moderate [≤14 drinks per week for men, ≤7 drinks/week for women] or heavy [>14 drinks/week for men, >7 drinks/week for women]), physical activity level (any weekly exercise vs. none), history of transient ischemic attack (TIA), coronary artery disease (CAD): defined as myocardial infarction by self-report or electrocardiogram (ECG) or history of coronary revascularization (stenting, coronary artery bypass surgery, or percutaneous transluminal coronary angioplasty)), atrial fibrillation (self-reported or by ECG), left ventricular hypertrophy (LVH) by ECG, body mass index (BMI) calculated from height and weight measurements, hypertension (systolic blood pressure>140 mmHg or diastolic blood pressure >90 mmHg on average of 2 measurements, or self-reported use of hypertension medication), dyslipidemia (total cholesterol ≥ 240 mg/dL, LDL ≥ 160, HDL ≤ 40 or self-reported lipid-lowering drug use), and diabetes (fasting glucose ≥126 mg/dL, nonfasting glucose ≥200 mg/dL, or self-reported use of diabetes medications). CRP>90th percentile was included as a covariate to control for inflammation. Aspirin, warfarin and statin use were determined by medication inventory. Incident stroke was ascertained via telephone follow-up every six months using the Questionnaire for Verifying Stroke-free Status [24] and verified by medical record review and adjudication by a panel of stroke experts. Stroke occurring after enrollment but prior to the most recent cognitive assessment was included as a covariate.

Nested Case Control Study

As previously described [18], we adapted a REGARDS case-cohort study sample drawn in 2011, which included an 1104-person cohort sample, to develop a nested case-control study of ICI. Among 17,630 participants without prevalent stroke, without baseline cognitive impairment based on the SIS, and who had sufficient follow up cognitive domain testing at the time of case selection in 2011, we identified an ICI case group of 495 participants as described in detail elsewhere[25]. Cases scored >1.5 SD below age, race, sex, and education adjusted predicted scores on at least 2 of the 3 cognitive domain tests at the most recently administered 3-test cognitive battery (median follow up 3.5 years). From the 1104-person cohort random sample, to develop a control group, we applied the same exclusion criteria as for case selection, excluding 83 participants with prevalent stroke, 108 with cognitive impairment on the first SIS, 306 with insufficient cognitive testing to determine whether they developed ICI, and 20 who developed ICI, leaving 587 controls for this nested case-control study.

Laboratory Analysis

Blood samples were collected at the baseline in-home visit. A draw tube containing 4.5 mmol/L EDTA, 0.15 KIU/L aprotinin, and 20 mol/L d-Phe-Pro-Arg-chloromethylketone (SCAT-1; Haematologic Technologies), designed to prevent in vitro clotting activation[26, 27], was included. This plasma was used for the current study. Samples were centrifuged near participant homes and shipped overnight to the University of Vermont core laboratory where they were re-centrifuged at 30,000 g-min, then stored at −80C. Case and control samples were analyzed together in random order so technicians were blinded to their status. D-dimer was measured using an automated immuno-turbidimetric assay (Liatest D-dimer, STA-R, Diagnostica Stago, interassay coefficient of variation (CV) 5–17%). Fibrinogen

antigen was measured using the BN-II nephelometer (Siemens, CV 3–8%). Factor VIII antigen and Protein C antigen were measured by ELISA (FVIII, Enzyme Research Laboratories, CV 4–7%, Protein C: Diagnostica Stago, CV 4–7%). Results above or below the detectable limit of the assay were recoded as at the detectable limit for statistical analysis. Validity of all assays performed on SCAT-1 plasma samples similarly processed and shipped overnight was confirmed previously [28]. There were 5.8% of the cases and 5.2% of the controls missing biomarker results mainly due to lack of sample availability. ApoE genotype was determined by TaqMan analysis of the 2 SNPs, rs429358 and rs4712 [29]. Haplotypes were reconstructed using the PHASE program [30], with ambiguous haplotypes coded as their most likely outcome if the probability was >85%. Cholesterol, glucose and CRP were previously measured in the entire REGARDS cohort [19, 31].

Statistical Analysis

Analyses were conducted in STATA 11.0 (College Station, TX). Because of the stratified nature of the control group, analyses were probability weighted and 95% confidence intervals were calculated using a Taylor series with finite population correction. Weights were based on the total number of participants who were eligible to become cases (N=17,630) and the probability of being selected for the cohort random sample for each stratum given ICI case eligibility.

Baseline characteristics were compared between cases and controls using weighted Pearson chi square tests (corrected for survey design with the second-order correction of Rao and Scott[32] and converted into an F statistic). Among controls, median biomarker levels were compared between blacks vs. whites and in stroke belt residents vs. non-residents using Wilcoxon rank-sum tests. Weighted logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (CI) of ICI for each biomarker, as an estimate of relative risk. Biomarkers were analyzed continuously, in quartiles, and as abnormal or normal, defining abnormal levels as those above the 90th percentile for factor VIII and fibrinogen, below the 10th percentile for protein C, and above the clinically used cutpoint of 0.50 μg/mL for D-dimer. Percentile/quartile cutpoints were calculated based on the weighted distribution in the cohort random sample. We examined burden of elevated biomarkers by assessing the association of ≥2 versus <2 abnormal biomarkers with ICI. When examined continuously, all four hemostasis biomarker predictors violated the assumption of a linear relationship between log odds ICI and predictor. Log transformation or quadratic terms did not resolve this violation, so continuously expressed concentrations were not investigated further; instead we used quartiles. Models were first carried out unadjusted, then adjusted for demographics, health behaviors, vascular risk factors, presence of ≥1 ApoE4 allele, incident stroke, and baseline warfarin, aspirin and statin use. A biomarker*race interaction term was tested in each model, and if significant, we stratified by race. Biomarker*region and biomarker*biomarker interaction terms were also examined. Because of concerns of co-linearity, models were run with and without CRP as a covariate. A sensitivity analysis was conducted excluding participants on warfarin at baseline.

We planned a mediation analysis for any biomarker that was associated with both ICI and had adverse levels in the stroke belt, however none of the biomarkers or the number of abnormal biomarkers met this criteria and the analysis was not performed.

Participants missing data on predictors or covariates were excluded from adjusted models, leaving 403 complete cases (82% of total) and 521 complete controls (83% of total). Those missing data were similar to those included except they were more likely to have low income and diabetes (supplemental table). Given the number of available cases and controls, considering biomarker concentrations >90th percentile as the risk factor, we had 80% power to detect an OR for ICI of 1.51.

Results

Weighted baseline characteristics of cases and controls are listed in Table 1. Because of the demographically-adjusted regression-based selection of ICI cases, cases and controls were similar by age, race, sex, and education. Cases were more likely to report lower income, current smoking, and alcohol abstention, and had higher prevalences of diabetes, hypertension, LVH, CAD, elevated CRP and obesity. Abnormal D-dimer, fibrinogen, and factor VIII, but not protein C, were more prevalent in cases than controls. Other characteristics in Table 1 were similar by case status. The small number of participants taking warfarin at baseline (38, 3.5%) had lower median D-dimer (0.27 vs. 0.43 μg/mL, p=0.004) and protein C (66% vs. 121%, p<0.001), but similar fibrinogen and factor VIII compared to nonusers. In the time between enrollment and the most recent cognitive assessment, 21 participants survived a stroke. Eight were controls and 13 were cases (p = 0.04 for difference).

Table 2 shows the OR of ICI for each hemostasis biomarker expressed in quartiles and as abnormal levels. Abnormal concentrations of D-dimer, fibrinogen, and factor VIII were all associated with ICI in unadjusted analysis. None of the hemostasis biomarkers were associated with ICI in fully adjusted analysis. Sensitivity analysis excluding CRP as covariate and excluding participants who reported warfarin use did not substantively change the results (data not shown).

In evaluation of differences in the association of hemostasis biomarkers with ICI risk by race, only the association of factor VIII >90th percentile with ICI appeared to differ by race (p interaction 0.06 in fully adjusted model; other p values for interaction >0.40 in unadjusted and adjusted models). While the adjusted OR of ICI for factor VIII >90th percentile vs lower was 1.76 (CI 0.88, 3.51) in whites and 1.05 (CI 0.43, 2.58) in blacks, this finding is likely spurious since for factor VIII in the top versus bottom quartile, the adjusted ORs were 2.31 (95% CI 0.95, 5.57) in blacks and 0.83 (95% CI 0.43, 1.58) in whites (p interaction 0.92).

Two-predictor models with hemostasis biomarkers and one covariate at a time were used to identify which factors were confounders of the association of hemostasis markers with ICI. For fibrinogen and factor VIII, income, diabetes, and CRP>90th percentile each decreased the β coefficient for both biomarkers by at least 10%, as did coronary heart disease for factor VIII. Income, diabetes, alcohol and BMI each decreased the β coefficient for D-dimer at

least 10%. In addition to individual confounders, we assessed groups of demographic, health behavior, or vascular risk factors in separate models (Table 3).

Considering D-dimer, fibrinogen and factor VIII (but not protein C since it wasn't associated with ICI), participants with ≥2 abnormal biomarkers versus <2, had an OR of ICI in unadjusted analysis of 2.17 (1.39, 3.37) and this was 1.90 (1.04, 3.51) in fully adjusted analysis. Most of the participants with ≥2 abnormal biomarkers had elevated D-dimer (96%); 49% had elevated fibrinogen and 55% had elevated factor VIII. These biomarkers were modestly correlated with each other (Spearman correlations 0.31–0.33). The interactions between D-dimer and factor VIII and between D-dimer and fibrinogen were not statistically significant on the multiplicative scale (p 0.20 and 0.36 respectively).

Residence in the stroke belt was associated with higher odds of ICI in unadjusted (OR 1.63, CI 1.25, 2.11) and fully adjusted (OR 1.60, CI 1.17, 2.40) analyses. Median D-dimer, fibrinogen, protein C, Factor VIII, and number of elevated hemostasis biomarkers ≥2 did not differ by region; consequently differences in biomarker levels were not mediators of this regional difference in ICI (data not shown). Further, there were no differences in the association of biomarkers with risk of ICI by region (all p> 0.10).

Discussion

In this prospective study, elevated concentrations of D-dimer, fibrinogen and factor VIII, but not low protein C, were modestly associated with ICI in unadjusted models. Associations did not differ by race or region. Other demographic and vascular risk factors accounted for the higher odds of ICI with these biomarkers as associations were attenuated in fully adjusted models.

Despite the lack of association of individual biomarkers with ICI risk, abnormal levels of ≥2 elevated biomarkers with ICI increased the risk nearly 2-fold in fully adjusted analyses. This essentially involved participants with elevated D-dimer plus at least one other elevated biomarker, suggesting that the burden of perturbed procoagulant balance relevant to risk of ICI cannot be detected with individual biomarkers. Our findings were similar to those of Gallacher and colleagues [4], who reported that concurrent elevation of fibrinogen, factor VIII, and PAI-1 were associated with a hazard ratio of 2.97 (CI, 1.38, 4.56) for incident VaD.

Residence in the stroke belt was associated with ICI in this analysis, similar to a previous report in REGARDS using a global cognitive test score[16]. The absence of higher levels of biomarkers in the stroke belt and no biomarker effect modification by region indicate that hemostasis biomarkers do not mediate the excess risk of ICI in the stroke belt. Despite limited literature suggesting lower protein C is associated with stroke [13], including in REGARDS [33], we did not see an association of lower protein C with ICI.

Fibrinogen is a marker of inflammation and a hypercoagulable state [34], and inflammation and hemostasis are tightly linked. Others reported that the association of fibrinogen and cognitive decline was stronger for VaD than for AD [4, 7, 10], suggesting mechanisms related to infarcts and neurostructural damage from atherosclerosis. However, in vitro and

mouse model evidence suggests β amyloid (Aβ), the hallmark protein of AD, interacts with fibrinogen to form abnormal fibrin structures [35, 36]. These abnormal fibrin/Aβ structures are deposited in the neurovasculature and are resistant to degradation [37], causing neurovascular inflammation and damage contributing to ICI in AD, and they can be inhibited pharmacologically in a mouse model [38]. Whether circulating levels of fibrinogen can reflect this biology is unclear.

The only prior study of factor VIII and ICI, to our knowledge, reported higher odds of VaD [4]. Several studies investigated the association of the related protein von Willebrand factor (vWF) with ICI, with primarily null findings [4, 6, 10]. Factor VIII and vWF are closely correlated as factor VIII binds vWF while circulating in blood and rapidly degrades when not bound. We did not measure vWF because field processing and shipping methods in REGARDS affected the validity of vWF assays[28]

We demonstrated multiple confounders of the association of hemostasis markers with ICI. Individually, income, diabetes, and CRP had large impacts. The D-dimer association was confounded by health behaviors and vascular risk factors. Factor VIII was most confounded by vascular risk factors. The association of fibrinogen and ICI remained apparent until demographic, health behavior, and vascular risk factors were all included in the model. Incremental decreases in most of the ORs with stepwise addition of covariates suggest that many of the covariates have a small but ultimately cumulative effect on the relationship between hemostasis biomarkers and ICI.

Differences between our findings and previous reports may be due to which covariates were accounted for. Many prospective studies reporting associations of fibrinogen with dementia or ICI did not adjust for socioeconomic factors [5, 7–9]. Stott and colleagues, who observed differences in rate of decline on several cognitive tests by D-dimer and fibrinogen level [6] adjusted for education, but not income, which was a strong correlate of ICI in our study, even when other socioeconomic variables including education were accounted for. Gallacher and colleagues [4] adjusted for social class and reported that fibrinogen, factor VIII, and PAI-1 were associated with incident VaD but not with non-VaD. Their study included only men within the Caerphilly locality in South Wales, England, which may also explain differences from our results.

Differences between our findings and other published literature may also be due to differences in definitions of the outcome or predictors. We could not determine the etiology of ICI in our study or classify it into clinical diagnoses. As some of the previous research identified associations of hemostasis biomarkers with VaD only [4, 7, 10], it is possible that this subgroup is too small within our ICI cases to identify individual biomarker associations. We studied biomarker levels as continuous, categorical, and dichotomous predictors. Only biomarkers above an established clinically significant cutpoint (>0.50 μg/mL for D-dimer) or at levels ≥90^{th} percentile of the distribution showed an association with ICI. Other previously published studies analyzed continuous [5, 7–9, 11] tertiles, [6] quintiles, [7, 10] or z-score [4] predictors. Previous prospective studies investigating D-dimer had mixed results, some reporting an association with VaD [6, 10] and ICI [11] and others finding no association. [4, 5] Additionally, most participants with 2 abnormal biomarkers had elevated

D-dimer and either high fibrinogen or high factor VIII. A biomarker score of positive D-dimer plus high levels of other hemostasis biomarkers was associated with ICI in a multivariable model, suggesting scores may be more useful in detecting associations than single biomarkers.

Limitations of this study include loss of statistical power in adjusted analyses due to missing covariates. Although complete cases did not differ substantially from incomplete cases, ~17% of the cases and controls were omitted from some of the fully adjusted analyses. We suspect that this limitation would only bias our findings to the null by reducing statistical power. We had 80% power to detect an OR for ICI of 1.51 in those with biomarker levels >90th percentile in adjusted analysis. The OR point estimates for fibrinogen and factor VIII in these models were 1.40 and 1.19, respectively. If these are the true effect sizes, a larger sample size is needed to confirm this finding. Results are generalizable to blacks and whites in the US, so might not apply to other groups. Finally, the findings regarding risk related to presence of ≥2 biomarkers should be interpreted cautiously given the lack of associations of individual biomarkers with ICI in the adjusted models, and since residual confounding due to unmeasured factors might partly explain this association.

Strengths of this study are inclusion of blacks and whites from a national cohort study and well-characterized vascular risk factors. The nested case-control design and baseline blood samples allowed prospective assessment of the association of biomarkers with incident ICI, reducing the likelihood of reverse causality to explain positive findings. Also, cases and controls were free from stroke and cognitive impairment on a screening tool at baseline. As stroke may be in the causal pathway between elevated hemostasis markers and ICI, we included participants who survived incident stroke and evaluated its effect in adjusted analyses.

In conclusion, individual hemostasis biomarkers were univariately associated with ICI, but these associations were not independent of other factors in adjusted models. Having at least 2 elevated biomarkers was associated with ICI after multivariable adjustment, suggesting individual markers have small but additive associations. Income, diabetes, and inflammation were important confounders of the relationship of individual biomarkers with ICI and should be taken into account in future studies. Neither individual hemostasis biomarkers nor biomarker scores mediated the association of stroke belt residence with ICI, and further studies are needed to elucidate the causes of this regional disparity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Essentials

- Cognitive disorders are increasing and vascular risk factors play a role in this.
- We performed a nested case control study of hemostasis biomarkers and cognitive impairment (CI).
- Higher baseline fibrinogen, factor VIII and D-dimer were related to incident CI over 3.5 years.
- 2+ abnormal markers (but not single ones) led to higher risk adjusted for other risk factors.

Table 1

Baseline Characteristics of Cognitive Decline Cases and Controls

Risk Factor % unless otherwise specified	Cases (N= 495)	Controls (N=587)*	p-value
Black Race	33	36	0.34
Age, Mean	64.6	64.1	0.34
Male Sex	41	43	0.55
Education			
≤ High school	33	32	
Some college	26	28	
≥College graduate	41	39	0.83
Stroke Belt Region	65	52	<0.001
Income			
< $20k	29	12	
$20k–$34k	20	22	
$35k–$74k	25	37	
≥ $75k	12	17	
Did not Answer	14	12	<0.001
Smoking Status			
Current	17	12	
Past	38	37	
Never	45	51	0.02
Alcohol Use			
Heavy	3	5	
Moderate	28	34	
None	69	61	0.01
Diabetes	29	19	<0.001
Hypertension	61	55	0.05
Dyslipidemia	61	58	0.44
History of Transient Ischemic Attack	6	4	0.16
Atrial Fibrillation	10	9	0.61
Left Ventricular Hypertrophy	12	7	0.03
History of Coronary Artery Disease	21	13	<0.01
BMI Category (kg/m2)			
<18.5 underweight	<1	<1	
>18.5 <25 normal	18	25	
≥25<30 overweight	34	35	
≥30 obese	47	40	0.03
Regular aspirin use	43	44	0.87
Statin use	34	31	0.34
Warfarin use	4	3	0.33
ApoE4 homo- heterozygous	32	31	0.69
C-reactive protein >90th percentile	14	10	0.04

Risk Factor % unless otherwise specified	**Cases (N= 495)**	**Controls (N=587)***	**p-value**
D-dimer >0.50 μg/mL	46	39	0.04
Fibrinogen >90th percentile	16	10	0.002
Factor VIII antigen, >90th percentile	15	10	0.01
Protein C antigen, <10th percentile	11	10	0.64
Number of biomarkers elevated			
0	45	53	
1	37	38	
2	14	7	
3	4	2	0.0001

* Weighted to analytic cohort, total N=17,630

Table 2

Hemostatic Factors and Odds Ratios of Incident Cognitive Impairment

		Odds Ratio (95% CI) ICI for Categories of Hemostasis Biomarkers			
Biomarker	**Model**	**Quartile 2 vs.1**	**Quartile 3 vs. 1**	**Quartile 4 vs. 1**	**Abnormal Value****
D-dimer, µg/mL	Unadjusted	1.03 (0.71, 1.52)	1.51 (1.06, 2.17)	1.32 (0.92, 1.89)	1.32 (1.02, 1.70)
	Adjusted*	0.94 (0.57, 1.54)	1.30 (0.80, 2.11)	1.02 (0.61, 1.71)	1.17 (0.83, 1.66)
Fibrinogen, mg/dL	Unadjusted	1.06 (0.74, 1.54)	1.09 (0.76, 1.50)	1.26 (0.8, 1.81)	1.83 (1.24, 2.71)
	Adjusted*	1.24 (0.78, 1.99)	1.02 (0.65, 1.74)	0.76 (0.45, 1.27)	1.40 (0.82, 2.41)
Factor VIII antigen, %	Unadjusted	0.71 (0.49, 1.04)	0.87 (0.60, 1.25)	1.37 (0.97, 1.93)	1.63 (1.11, 2.38)
	Adjusted*	0.56 (0.34, 0.90)	0.74 (0.46, 1.20)	0.94 (0.50, 1.60)	1.19 (0.71, 1.97)
Protein C antigen, %	Unadjusted	0.84 (0.59, 1.19)	0.97 (0.68, 1.38)	0.77 (0.52, 1.09)	1.10 (0.73, 1.65)
	Adjusted*	0.78 (0.49, 1.24)	1.17 (0.73, 1.87)	0.75 (0.45, 1.24)	0.94 (0.50, 1.76)

*Adjusted for age, race, sex, education, income, region, smoking, alcohol use, CAD, atrial fibrillation, hypertension, diabetes, dyslipidemia, LVH, history of TIA, BMI, CRP>90th percentile, aspirin use, statin use, warfarin use, presence of at least 1 Apoe4 allele

**D-dimer >0.50 µg/mL, factor VIII >90th percentile (168%), fibrinogen >90th percentile (502 mg/dL), protein C <10th percentile (95%)

D-dimer Q1≤0.23 µg/mL, Q2=0.26–0.37 ug-mL, Q3=0.38–0.74 µg/mL, Q4≥0.75µg/mL

Factor VIII Q1≤91%, Q2=91–109%, Q3=109–134%, Q4≥134%

Fibrinogen Q1≤331mg/dL, Q2=332–381 mg/dL, Q3=382–441 mg/dL, Q4≥442 mg/dL,

Protein C Q1≤108%, Q2=108–122%, Q3=122–136%, Q4≥136%

Table 3

Analysis of Factors that Confounded Associations of Hemostatic Factors with Cognitive Impairment

	Odds Ratio (95% CI) in Models Adjusted for Different Groups of Risk Factors							
Biomarker	**Crude**	**Crude+DEM1**	**Crude +DEM2**	**Crude + DEM 1+ DEM2**	**Crude + HB**	**Crude + VRF**	**Crude + DEM1 +DEM2 + HB**	**Crude + DEM +HB +VRF**
D-dimer, > 0.50 ug/mL	1.32 (1.02, 1.70)	1.27 (0.97, 1.66)	1.32 (1.00, 1.74)	1.39 (1.04, 1.87)	1.21 (0.92, 1.59)	1.20 (0.88, 1.62)	1.26 (0.92, 1.69)	1.12 (0.79, 1.58)
Fibrinogen > 90th percentile	1.83 (1.24, 2.71)	1.59 (1.04, 2.43)	1.90 (1.27, 2.84)	1.71 (1.11, 2.63)	1.62 (1.06, 2.48)	1.66 (1.03, 2.68)	1.51 (0.95, 2.38)	1.35 (0.81, 2.26)
Factor VIII > 90th percentile	1.63 (1.11, 2.38)	1.62 (1.11, 2.39)	1.74 (1.17, 2.59)	1.71 (1.14, 2.55)	1.56 (1.04, 2.34)	1.35 (0.87, 2.09)	1.57 (1.02, 2.41)	1.14 (0.69, 1.89)

DEM1= demographic factors: income, region

DEM2= demographic factors: age, race, sex, education

HB= health behaviors: smoking, alcohol, exercise

VRF= vascular risk factors: CAD, atrial fibrillation, hypertension, dyslipidemia, diabetes, LVH, history of TIA, BMI, CRP>90th percentile, aspirin use, statin use, warfarin use, incident stroke.