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Haemostasis Biomarkers and Risk of Intracerebral Haemorrhage in the REasons for Geographic and Racial Differences in Stroke Study

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Abstract

Background: Pathologic alterations in haemostasis cause bleeding disorders, but it is unknown if variation within the normal range relates to intracerebral haemorrhage (ICH) risk.

Objectives: To assess the prospective associations of haemostasis biomarkers with ICH risk.

Materials and Methods: The REasons for Geographic and Racial Differences in Stroke study (REGARDS) recruited 30,239 U.S. individuals >45 years. ICH was ascertained through biannual telephone contact and review of deaths followed by medical record evaluation. Haemostasis biomarkers (factor VIII (FVIII), factor IX (FIX), factor XI (FXI), fibrinogen, protein C, and D-dimer) were measured in a case cohort study consisting of ICH and a 1,104 person cohort random sample. The hazard ratio (HR) and 95% confidence interval (CI) by biomarker were estimated using Cox models and adjusted for ICH risk factors. Individuals with a prior history of stroke, ICH or on warfarin were excluded.

Results: Over a median 5.8 years 66 ICH occurred. Fibrinogen, FVIII, FXI, and protein C were not associated with ICH risk in any analysis. Lower FIX increased risk of ICH with the bottom versus the top tertile of FIX associated with an HR of 5.68 (95% CI 2.30, 14.05). D-dimer demonstrated a non-linear relationship with a potential threshold effect with increased risk only in the top 5th percentile (HR 3.22; 95% CI 1.01, 10.31; $p_{\text{non-linear}} = 0.04$).

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Addendum

N.A. Zakai, N.C. Olson, S.E. Judd, D.O. Kleindorfer, B.M. Kissela, G. Howard, and M. Cushman designed the research. N.A. Zakai and N.C. Olson performed statistical analyses. N.A. Zakai and G. Howard obtained funding. N.A. Zakai drafted the manuscript, and N.A. Zakai, N.C. Olson, S.E. Judd, D.O. Kleindorfer, B.M. Kissela, G. Howard, and M. Cushman made critical revisions to the manuscript and helped interpret the data.

Disclosure of Conflicts of Interest

The authors declare no competing financial interests.

Conclusions: Low FIX levels within the normal range were associated with increased ICH risk. These data suggest non-pathologic alterations in haemostasis impact intracranial bleeding risk.

Keywords

Cerebral Haemorrhage; Cohort Studies; Continental Population Groups; Epidemiology; Haemostasis

Introduction

Primary intracerebral haemorrhage (ICH) is an uncommon (~10-15% of strokes, or 21 per 100,000 person-years follow-up) but devastating subtype of stroke in the developed world(1, 2). With a few exceptions(1, 3), most studies of ICH risk factors are retrospective(4–6). Derangements of haemostasis have mostly been studied in relation to risk for thrombotic disorders, with only severe deficiencies thought related to pathologic bleeding in disorders such as fibrinogen deficiency or haemophilia A, B, and C (FVIII, FIX, and FXI deficiencies respectively)(7–9). Concentrations of haemostasis biomarkers vary 2-3 fold on a population level, however whether variation within the ‘normal’ range is associated with increased risk of ICH is unknown.

The United States (U.S.)-based REasons for Geographic and Racial Differences in Stroke (REGARDS) study with its large, geographically dispersed, and contemporary participant recruitment offers the opportunity to prospectively assess risk factors for ICH in blacks and whites(10, 11). We hypothesized that basal lower levels of procoagulant proteins (fibrinogen, factor VIII, factor IX, and factor XI), higher levels of anticoagulant proteins (protein C) and the haemostasis activation marker (D-dimer), in individuals without a diagnosed bleeding disorder, would be associated with an increased risk of future ICH.

Materials and Methods

Cohort

REGARDS enrolled 30,239 individuals 45 years old from the contiguous U.S. from 2003-07. REGARDS aimed to recruit a half male, half black cohort; and geographically, half from the Southeast or ‘stroke belt’ (North Carolina, South Carolina, Georgia, Alabama, Mississippi, Tennessee, Arkansas, and Louisiana), with 25% of individuals from the coastal plains of North Carolina, South Carolina, and Georgia (stroke buckle). The final cohort was 55% female, 41% black, and 56% resided in the Southeast. Exclusion criteria were medical conditions preventing long-term participation, active cancer or cancer treatment within the past year, resident in or on the waiting list for a nursing home, inability to communicate in English, or participant self-report of race other than black or white. Participants were contacted by telephone, and after verbal informed consent participated in a computer assisted telephone interview gathering demographic and risk factor data. Participants then underwent an in-home visit where anthropomorphic data, medication inventory, fasting phlebotomy, and written informed consent was performed by trained technicians (Examination Management Systems Incorporated, Irving, Texas)(10, 11). The study was approved by the institutional review boards of all participating institutions.

Definitions

Prevalent stroke, cardiovascular disease (defined as coronary heart disease, revascularization, or peripheral arterial disease) was based on participant self-report or electrocardiogram evidence of a prior myocardial infarction. Atrial fibrillation was defined as participant self-report or by electrocardiogram at the in-home exam and left ventricular hypertrophy by electrocardiogram criteria. Diabetes mellitus was defined as fasting glucose ≥ 126 mg/dL, nonfasting glucose ≥ 200 mg/dL or self-reported use of diabetes medications. Systolic blood pressure was the average of two seated measures after a 5 minute rest. Use of antihypertensive medications, regular aspirin, and warfarin was defined by participant self-report. Hypertension was defined as SBP ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or self-reported use of antihypertensive medications. Smoking and alcohol use were ascertained from the initial telephone interview. Alcohol use was modelled as 0, 1-7, and >7 drinks / week and smoking as current smoking or not currently smoking.

Laboratory

Phlebotomy was performed after a 10-12 hour fast in the morning of the in-home visit(12). EDTA plasma samples were centrifuged locally and shipped overnight to the study laboratory at the University of Vermont where they were re-centrifuged and stored at -80°C . FVIII, FIX, and FXI were measured using an enzyme linked immunosorbent assay (Enzyme Research Laboratories, South Bend, Indiana, U.S.) and Protein C antigen by an Enzyme Linked Immunosorbent Assay (Diagnostic Stago, Parsippany NJ) all reported as the percent (%) of normal pooled plasma. Interassay coefficients of variation were 4-15%. D-dimer was measured using an immunoturbidometric assay on the STAR analyzer and reported in $\mu\text{g/mL}$ (Diagnostic Stago, Parsippany NJ) with an interassay coefficient of variation of 5%-17%. Fibrinogen antigen was measured using an immunonephelometric assay using the Siemens Dade Behring BN II Nephelometer (Siemens Healthcare Deerfield, IL) with an interassay coefficient of variation of 3%-8%.

ICH Outcome

Participants were followed for incident stroke (both ischemic and ICH) via biannual telephone contact with participants or proxies using previously reported methods(3, 10, 11). Medical records were retrieved and centrally reviewed by an adjudication committee, including those from all deaths. Adjudication was based on the World Health Organization's definition of stroke and/or imaging consistent with stroke. ICH was determined by review of medical records and imaging reports by at least two members of the adjudication committee based on the World Health Organizations definition of stroke and with no evidence of an antecedent ischemic stroke with secondary hemorrhage.

Statistical Analyses

We performed a stratified case-cohort analysis using methods proposed by Prentice(13–16). This methodology allows the cohort random sample (CRS) to serve as the control group for multiple endpoints of interest and allows use of our time-to-event data. By employing a stratified CRS, we improved power to detect associations in age, sex, and race strata of interest (i.e. elderly black men). Briefly, individuals in the CRS contributed person-time to

the analysis until they were censored or 1 second prior to an ICH event. All ICH cases contributed person-time in the analysis from 1 second prior to an ICH event to the ICH event (including those in the CRS). Biomarkers of interest were measured in incident ICH cases and a 1,104-person stratified CRS. Individuals were censored at death, loss to follow-up, at the time of an ischemic stroke, or on 1 September 2011 (whichever occurred first). In this analysis, participants with baseline stroke or baseline warfarin use were subsequently excluded as cases or non-cases. Individuals with missing covariates were dropped from analysis. Continuous variables with a substantial skewing by visual inspection were natural-log transformed. Quantiles of biomarkers were defined using the CRS accounting for sample weighting. Differences in means between the CRS and ICH cases were determined using weighted analysis of variance and categorical variables were compared using the Rao-Scott χ^2 statistic. Correlations between haemostasis biomarkers were assessed using Pearson's r. We employed Cox proportional hazards models to estimate the hazard ratios (HR) of biomarkers for ICH with robust sandwich estimators to calculate 95% confidence intervals (CI) accounting for sample weighting. Two models were used; Model 1 adjusted for demographic variables (age (as a continuous variable), sex, race, region (southeast versus the rest of the U.S.), and an age by race interaction term (due to a previously identified age by race interaction(3, 10)). Model 2 adjusted further for stroke risk factors (aspirin use, systolic blood pressure (as a continuous variable), diabetes, current smoking, cardiovascular disease, atrial fibrillation, left ventricular hypertrophy, antihypertensive medication use, and alcohol use. In exploratory models, we tested for interactions between haemostasis biomarkers with race and sex using a cross product term. Biomarkers were modelled as percentiles (by tertiles and below the 10th and above the 90th percentile compared to remainder of the population) and per SD increments. We tested for non-linearity in the association of biomarkers with ICH by adding a quadratic term into the model, and if present performed further analyses to determine the shape of association of the biomarker with ICH. Haemostasis biomarkers were modelled based on hypothesized direction of increased risk (lower levels of fibrinogen, FVIII, FIX, and FXI and higher levels of protein C and D-dimer). Sensitivity analyses excluding the bottom or the top 2.5th percentile of biomarker levels were performed to assess the potential impact of extreme biomarker levels. We conducted an additional analysis assessing whether the number of biomarkers (of FVIII, FIX, FXI, or fibrinogen) in the bottom tertile (modelled as 1, 2, 3, or 4) increased ICH risk adjusting for risk factors in Model 2. All analyses were done using SAS 9.3 (Gary, North Carolina). All co-authors had access to the data.

Results

Over a median of 5.8 years, there were 73 ICH events. Excluding individuals on warfarin or with a history of prior stroke left 66 ICH events and 986 CRS participants. Table 1 presents the baseline characteristics of the CRS (in those free of ICH at follow-up) and the ICH cases. Individuals with ICH were older, more likely to be male, use aspirin, have higher systolic blood pressure, have left ventricular hypertrophy and hypertension, have baseline cardiovascular disease, and reported higher alcohol use than individuals in the CRS. Conversely, individuals with ICH were less likely to have atrial fibrillation than those without ICH (although this finding is of uncertain significance as individuals on warfarin at

baseline were excluded from analyses). Individuals with ICH had lower baseline levels of FIX (97% vs. 104%) and protein C (116% vs. 122%) and higher D-dimer (0.51 µg/mL vs. 0.43 µg/mL) than individuals without ICH (Table 1).

Table 2 presents the distributions and SD of the biomarkers as well as the number of individuals with biomarker measures available. Protein C and FIX were moderately correlated (correlation coefficient 0.48; $p < 0.001$) with weak correlations (correlation coefficients all ~ 0.3) between FVIII and D-dimer, FIX and FXI, FXI and protein C, and between fibrinogen and all the haemostasis biomarkers except protein C (Table 3).

Table 4 presents associations of the haemostasis biomarkers with ICH. The results of the demographic-adjusted models (Model 1) were similar to the cardiovascular risk factor adjusted models (Model 2) with no associations between fibrinogen, FVIII, or FXI and ICH risk. There was a strong and consistent association of lower FIX and more complex associations of protein C and D-dimer with ICH risk. After adjustment for stroke risk factors, each SD lower FIX was associated with an increased ICH hazard of 1.97 (95% CI 1.47, 2.65). Compared to those in the top tertile of FIX, those in the middle and lowest tertiles (corresponding to values of 94–112% and $< 94\%$) had ~ 2 and ~ 6 -fold increased risk of ICH. The global p -value for the association of FIX tertiles with ICH was < 0.001 . Excluding individuals in the bottom 2.5th percentile of the distribution ($< 66\%$) did not meaningfully change the association of the first vs. the third tertile of FIX with ICH (HR 5.68; 95% CI 2.29, 14.10).

Higher protein C was associated with lower ICH risk in Model 2 (Table 4). As protein C and FIX were moderately correlated (Table 2), we added FIX into the model with protein C. For the continuous (per SD higher) analysis, the association of protein C with ICH became null when factor IX was added into Model 2 (HR 1.01; 95% CI 0.66, 1.55); there was no change in the association of FIX with ICH when protein C was added to Model 2 (HR 1.99 per SD lower; 95% CI 1.30, 3.06). When FIX was added to models containing protein C in the tertile analysis, the association of protein C with ICH was lost with no change in the association of FIX tertiles with ICH (data not shown).

D-dimer was not significantly associated with ICH modelled as a continuous variable or as tertiles. The p -value for the quadratic term in the continuous analysis was borderline significant ($p = 0.04$) suggesting a potential non-linear association. To explore a non-linear association we divided D-dimer into tertiles, and then added categories for the top and bottom 5% of the distribution. In model 2 (middle tertile as the reference), the HR for haemorrhagic stroke for the bottom 5% was 1.74 (95% CI 0.18, 16.90), first tertile (5th – 33rd percentile) 1.79 (95% CI 0.79, 4.03), top tertile (66th – 95th percentile) 0.99 (95% CI 0.41, 2.36), and for the top 5% was 3.22 (95% CI 1.01, 10.31).

There were no significant interactions between the haemostasis biomarkers and race or sex (all $p_{\text{interactions}} > 0.10$) and sensitivity analyses removing individuals at the bottom or top 2.5% of biomarker distributions did not change the interpretation or significance of the results, and the p -value for the quadratic term in the continuous analysis was non-significant for all biomarkers except D-dimer (data not shown).

To assess whether there was any additional impact on ICH risk by low levels of multiple haemostasis biomarkers, we evaluated whether the number of biomarkers with low levels was associated with ICH risk. Biomarkers (fibrinogen, FVIII, FIX, and FXI) in the lowest third of the distribution (33.3rd percentile, Table 2) were included in Model 2 and assessed per every 1 additional biomarker added and by the total number of biomarkers with low levels (1, 2, 3, or 4 versus none). The initial analysis demonstrated that per each additional low haemostasis biomarker there was an increased risk of ICH (HR 1.32; 95% CI 1.05, 1.66) and an increasing ICH HR for the total numbers of low haemostasis biomarkers in the model compared with no low levels (Table 5). However, these findings were driven solely by the probability of FIX being low. When we removed FIX from the biomarker score, the associations became null; per 1 additional low biomarker, the HR was 1.11 (95% CI 0.82, 1.51) with a similar finding in the categorical analysis (Table 5).

Discussion

In REGARDS, lower FIX and D-dimer in the top 5% were associated with increased risk of ICH. To our knowledge, these findings are novel and have not been reported in healthy populations.

Variation of FIX within the ‘normal’ population range is not known to increase bleeding risk. Genetic FIX deficiency, or haemophilia B (an X-linked bleeding diathesis with factor IX levels <15%), is associated with an increased risk of ICH, with increasing ICH risk with lower levels of FIX(7, 17). While female haemophilia B carriers (baseline FIX levels ~50%) are reported to have increased minor bleeding compared to non-carriers, haemophilia B carriers are not known to have, nor have been assessed for, an increased ICH risk, and in fact FIX levels >60% are considered sufficient for normal haemostasis(7, 18). Undiagnosed haemophilia B carriers are unlikely to contribute to our findings as the carrier frequency in females is low (~1 in 15,000 individuals(7)), we found no difference in the association of FIX with ICH by sex, and excluding individuals with FIX concentrations less than the 2.5th percentile (<66%) did not change our results. Genome wide association studies of ICH have not revealed associations of coagulation-related genes with ICH risk, however these studies usually exclude chromosome X, the location of FVIII and FIX(19).

Insight into the association of coagulation defects with ICH risk is provided by information on the differential association of anticoagulant medications with ICH. Warfarin, a vitamin K antagonist, exerts an anticoagulant effect via interfering with the posttranslational carboxylation of glutamate residues on FII (thrombin), FVII, FIX, and FX(20) (hence resulting in functional deficiencies of these proteins). In clinical trials of warfarin versus oral direct thrombin inhibitors (dabigatran(21)) or FX inhibitors (rivaroxaban(22), apixaban(23), or edoxaban(24)), warfarin consistently demonstrated a ~2-fold increased risk of ICH despite similar incidences of overall bleeding and thrombotic outcomes. In the Cardiovascular Health Study with 106 ICH events, FVII was not associated with ICH risk(25). Given haemophilia B (genetic FIX deficiency) and iatrogenic reduction of FIX activity (via warfarin) increases ICH risk more than inhibition of other coagulation proteins, our data support that lower FIX levels are causally related to increased ICH risk. An alternate hypothesis could be that reduction in multiple haemostasis biomarkers could relate

to ICH risk rather than a single haemostasis biomarker, however we did not see this in our analysis including low fibrinogen, FVIII, FIX, and FXI in the same model.

The association of D-dimer with ICH was potentially more complex. Circulating D-dimer is formed after three in-vivo enzymatic processes. First, fibrinogen is cleaved by thrombin to fibrin which polymerizes (forming the scaffold for a blood clot). The fibrin clot is then stabilized via cross-linking by FXIII and finally plasmin remodels the clot through lysis, releasing cross-linked fibrin fragments (D-dimer)(9). Clinically, higher D-dimer is seen in diseases associated with thrombosis, inflammation, or, more rarely, increased fibrinolysis(9, 26). In the European Prospective Investigation into Cancer and Nutrition-Italy cohort(27), higher baseline D-dimer levels were strongly prospectively associated with stroke and haemorrhagic stroke – which was the same direction as our findings. Comparability of these findings with REGARDS and other cohorts is difficult as individuals with atrial fibrillation or on anticoagulants could not be identified in the Italian study.

We hypothesized that high baseline protein C levels might be associated with increased ICH risk as exogenous administration of recombinant activated protein C (drotrecogin alfa) in severe sepsis patients was associated with an increased risk of bleeding in clinical trials (3.5% vs. 2.0%, $p = 0.06$)(28). Deficiency of protein C increases the risk of thrombosis, but there is no known bleeding phenotype associated with high endogenous levels of protein C(29, 30). While in our initial analysis, high protein C was associated with a *decreased* risk of ICH (opposite to the hypothesized effect), this was explained by the moderate correlation between protein C and FIX.

The lack of association of FVIII with ICH was surprising as congenital FVIII deficiency (haemophilia A) is an X-linked bleeding disorder associated with spontaneous ICH with increasing risk with lower FVIII levels(1, 17, 31). Further, female carriers of haemophilia A (FVIII levels ~50%) have increased bleeding compared with age matched controls, although an increased risk of ICH has not been reported (though also has not been assessed) in this population(18). The haemophilia literature does not provide insights to differential bleeding rates in those with FVIII or FIX deficiency as these conditions are usually reported together(17, 31). In one case series, 106 ICH in 1,410 individuals with FVIII or IX deficiency, ICH presented at an earlier age in individuals with FIX deficiency(32). Consistent with our findings, in a prior analysis of two pooled cohorts, neither FVIII nor its carrier protein von Willebrand factor were associated with ICH risk(1).

Low fibrinogen and factor XI were not associated with ICH risk in REGARDS. Fibrinogen deficiency is rare with severe bleeding phenotypes only at undetectable levels of fibrinogen(33, 34). In a pooled analysis of two cohorts, higher fibrinogen was associated with an increased risk of ICH (an association opposite to that hypothesized based on the physiologic function of fibrinogen), however there was significant heterogeneity of the association between cohorts(1). FXI deficiency (haemophilia C) is usually a mild bleeding disorder even in individuals with severely reduced FXI levels (<15%)(33). Further, in phase II studies of a novel FXI antisense oligonucleotide which reduces FXI levels, the incidence of thrombosis events was similar to enoxaparin (a factor Xa inhibitor) while bleeding was non-significantly lower (3% versus 8%)(35).

The main limitation of this study is power as we only had 60 cases of ICH with biomarkers measured. This is due to ICH being a rare event and the need for large cohort studies with long-follow-up to capture ICH events and prospective risk factor data. We maximized our power using a stratified case-cohort approach and were able to take advantage of our time-to-event information. Due to the strong association of FIX with ICH, we were adequately powered for this analysis, however we cannot rule out small to moderate associations of the other biomarkers with ICH or threshold effects at the extremes of the normal distribution. Specifically, for an exposure dichotomized at the median, with 60 events we would have 90% power to detect a hazard ratio of 2.31, and 80% power to detect a hazard ratio of 2.06. Due to the large number of statistical tests, findings of borderline statistical significance such as those seen with D-dimer, should be taken in context. Another limitation is the potential for unmeasured confounders; analysing individual coagulation proteins in isolation does not replicate the complex interactions between blood proteins, cellular blood components, and blood vessel endothelium which promote haemostasis(36). Participants misreporting warfarin use would unlikely affect our analyses as therapeutic warfarin would result in factor IX levels <30% and excluding individuals with factor IX levels <66% did not impact our results. Biomarkers were measured prior to the ICH events, often years prior to the events. Haemostasis is dynamic, and we cannot determine whether low factor levels immediately preceding the ICH event helped trigger the event. In spite of this limitation, prospective risk factor ascertainment (including biomarkers) is one of the greatest strengths of this study. We found a strong and robust association of FIX with ICH and perhaps a non-linear association of D-dimer with ICH in a well characterized, diverse cohort with prospectively ascertained risk factors.

In conclusion, lower levels of FIX are strongly and consistently associated with increased risk of future ICH independent of other ICH risk factors, D-dimer may have a threshold effect with increased ICH risk only at the highest levels. Fibrinogen, FVIII, FIX, and protein C either have weak or no associations with ICH risk. These data offer potential mechanisms behind differential association of various anticoagulants on ICH risk and guide drug development to help reduce ICH risk. Further, these data reinforce the notion that, at least for factor IX, the clinical phenotype of bleeding is a continuum of risk and not fixed to a specific threshold. Clinicians and researchers must rethink the definition of bleeding disorders, especially when evaluating individuals with ICH or at risk for ICH. The prior paradigm of fixed biomarker thresholds to define bleeding disorders is slowly falling and a more nuanced approach to haemostasis reflecting gradation of risk is urgently needed.

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Extra Table

What is known on this topic	What this paper adds
The impact of ICH on individuals can be devastating	Lower factor IX levels in the 'normal' range are strongly associated with an increased risk of ICH
Prospective study of risk factors for ICH is limited as this is a rare event on a population level	D-dimer in the top 5% of the distribution may increase the risk for ICH
Variation in haemostasis within population norms is not thought to increase pathologic bleeding	Factors VIII, XI, protein C, and fibrinogen were not associated with ICH risk.

Table 1:

Associations of Cardiovascular Risk Factors and Haemostasis Biomarkers with Incident Intracerebral Haemorrhage

Risk Factor	ICH* N = 66	Non-Cases* N = 986	P
Age (mean in years, SD)	67 (9)	65 (3)	<0.001
Male (n, %)	41 (62%)	481 (44%)	<0.001
Black Race (n, %)	29 (44%)	490 (41%)	0.15
Region (n, %)	-	-	-
Belt	20 (30%)	332 (34%)	-
Buckle	12 (18%)	177 (18%)	0.26
Rest of U.S.	34 (51%)	474 (47%)	-
Aspirin (n, %)	35 (53%)	408 (44%)	<0.001
Systolic Blood Pressure (mean mmHg, SD)	135 (19)	127 (17)	<0.001
Diabetes (n, %)	17 (26%)	207 (22%)	0.08
Current Smoking (n, %)	10 (15%)	138 (14%)	0.35
Cardiovascular Disease (n, %)	17 (26%)	173 (16%)	<0.001
Atrial Fibrillation (n, %)	3 (5%)	61 (7.3%)	0.03
Left Ventricular Hypertrophy (n, %)	12 (18%)	92 (8%)	<0.001
Hypertension (n, %)	38 (61%)	496 (51%)	<0.001
Alcohol use (n, %)	-	-	-
None	37 (65%)	621 (70%)	-
1-7 Drinks / week	12 (21%)	180 (23%)	0.001
>7 Drinks / week	8 (14%)	57 (8%)	-
Fibrinogen (mean mg/dL, SD)	393 (113)	392 (106)	0.76
Factor VIII (mean %, SD)	120 (56)	119 (45)	0.72
Factor IX (mean %, SD)	97 (20)	104 (22)	<0.001
Factor XI (mean %, SD)	108 (38)	107 (26)	0.97
Protein C (mean %, SD)	116 (26)	122 (24)	<0.001
D-dimer (geometric mean ug/mL, IQR)	0.51 (0.26, 1.02)	0.43 (0.23, 0.77)	0.005

* Individuals on warfarin at baseline or with a prior history of stroke were excluded. Percentiles in the cohort random sample were weighted by sampling frequency. Cardiovascular disease was defined as a past history of coronary heart disease or peripheral vascular disease.

Table 2:

Biomarker Distributions and Percentiles in the Cohort Random Sample

Biomarker (units)	Number Measured CRS (n = 986)	Cases (n = 66)	Standard Deviation	Percentile					
				2.5 th	10 th	33.3 rd	50 th	66.6 th	97.5 th
Fibrinogen (mg/dL)	938	60	106	84	289	353	387	427	605
Factor VIII (%)	939	60	45	60	77	99	112	129	220
Factor IX (%)	939	60	22	66	79	94	103	112	149
Factor XI (%)	939	60	26	63	78	95	105	117	161
Protein C (%)	940	60	24	82	96	112	120	130	169
D-dimer (µg/mL)	934	59	0.89*	0.08	0.16	0.29	0.40	0.62	2.64

* Log transformed

Table 3:

Correlation Coefficients among Haemostasis Biomarkers in the Cohort Random Sample *

Biomarker (Pearson's Correlation Coefficient, p-value)						
	Fibrinogen	Factor VIII	Factor IX	Factor XI	Protein C	Log D-dimer
Fibrinogen	-	0.34 <0.001	0.34 <0.001	0.25 <0.001	0.00 0.96	0.28 <0.001
Factor VIII	-	-	0.21 <0.001	0.15 <0.001	-0.04 0.16	0.31 <0.001
Factor IX	-	-	-	0.39 <0.001	0.49 <0.001	0.23 <0.001
Factor XI	-	-	-	-	0.35 <0.001	0.13 <0.001
Protein C	-	-	-	-	-	0.01 0.78

* White: No Correlation. Light Grey: Weak Correlation (Pearson's Correlation Coefficient ~0.3). Medium Grey: Moderate Correlation (Pearson's Correlation Coefficient ~0.5)

Table 4:

Associations of Inflammation and Haemostasis Biomarkers with Intracerebral Haemorrhage (Hazard Ratio, 95% Confidence Intervals)

Biomarker*	Model 1[†]	Model 2[‡]
Fibrinogen	-	-
Per SD Lower	0.99 (0.74, 1.32)	1.04 (0.72, 1.49)
Below versus above the 10 th percentile	1.08 (0.43, 2.69)	1.08 (0.35, 3.31)
Above versus below the 90 th percentile	1.37 (0.62, 3.02)	1.03 (0.37, 2.89)
Tertiles		-
1	0.92 (0.49, 1.73)	0.88 (0.39, 1.96)
2	0.81 (0.43, 1.53)	0.77 (0.39, 1.64)
3	Reference	Reference
Factor VIII	-	-
Per SD Lower	0.92 (0.65, 1.30)	0.87 (0.61, 1.25)
Below versus above the 10 th percentile	1.84 (0.87, 3.89)	1.26 (0.44, 3.65)
Above versus below the 90 th percentile	1.57 (0.73, 3.35)	1.86 (0.80, 4.33)
Tertiles		-
1	1.30 (0.70, 2.40)	1.10 (0.53, 2.29)
2	0.93 (0.48, 1.80)	1.08 (0.52, 2.27)
3	Reference	Reference
Factor IX	-	-
Per SD Lower	1.67 (1.24, 2.26)	1.97 (1.47, 2.65)
Below versus above the 10 th percentile	2.35 (1.22, 4.53)	2.87 (1.35, 6.11)
Above versus below the 90 th percentile	0.76 (0.28, 2.05)	0.41 (0.11, 1.48)
Tertiles [§]	-	-
1	3.43 (1.62, 7.24)	5.68 (2.30, 14.05)
2	1.76 (0.79, 3.89)	2.05 (0.75, 5.60)
3	Reference	Reference
Factor XI	-	-
Per SD Lower	0.97 (0.70, 1.34)	1.03 (0.72, 1.46)
Below versus above the 10 th percentile	1.48 (0.74, 2.98)	1.30 (0.51, 3.34)
Above versus below the 90 th percentile	1.51 (0.67, 3.40)	0.79 (0.25, 2.48)
Tertiles	-	-
1	1.18 (0.62, 2.23)	1.26 (0.58, 2.71)
2	0.78 (0.39, 1.55)	0.86 (0.35, 2.10)
3	Reference	Reference
Protein C	-	-
Per SD Higher	0.74 (0.54, 1.02)	0.66 (0.48, 0.90)
Below versus above the 10 th percentile	1.78 (0.96, 3.31)	1.86 (0.89, 3.98)
Above versus below the 90 th percentile	0.93 (0.35, 2.46)	0.50 (0.11, 2.20)
Tertiles	-	-

Biomarker [*]	Model 1 [†]	Model 2 [‡]
1	Reference	Reference
2	0.68 (0.36, 1.27)	0.76 (0.35, 1.64)
3	0.51 (0.25, 1.00)	0.35 (0.14, 0.87)
D-dimer	-	-
Per log SD Higher ^{**}	1.06 (0.76, 1.49)	0.97 (0.63, 1.49)
Below versus above the 10 th percentile	2.42 (1.04, 5.64)	2.44 (0.87, 6.87)
Above versus below the 90 th percentile	1.13 (0.50, 2.55)	1.26 (0.46, 3.45)
Tertiles	-	-
1	Reference	Reference
2	0.67 (0.34, 1.31)	0.56 (0.25, 1.24)
3	1.00 (0.53, 1.88)	0.69 (0.31, 1.54)

^{*} Percentiles of biomarkers defined in Table 2.

[†] **Model 1:** Adjusted for age (continuous), sex, race, region (southeast vs. rest of the U.S.), age*race interaction term.

[‡] **Model 2:** Adjusted for variables in Model 1 plus regular aspirin use, systolic blood pressure (continuous), diabetes, current smoking, baseline cardiovascular disease, baseline atrial fibrillation, baseline left ventricular hypertrophy, baseline use of antihypertensive medications, alcohol use (0, 1-7, and >7 drinks/week).

[§] Global p-value <0.001, non-significant for other biomarkers.

^{**} P_{non-linearity} = 0.04, non-significant for other biomarkers.

Table 5:

Association of Multiple Low Haemostasis Biomarkers with Intracerebral Haemorrhage (Hazard Ratio; 95% Confidence Interval)*

	Biomarkers Included in Score (HR; 95% CI)	
	Fibrinogen, FVIII, FIX, FXI	Fibrinogen, FVIII, FXI
Per 1 additional low biomarker [†]	1.32 (1.05, 1.66)	1.11 (0.82, 1.51)
Number of low biomarkers (reference 0 biomarkers low) [†]		
1 biomarker low	1.78 (0.75, 4.22)	1.38 (0.65, 0.65)
2 biomarkers low	2.64 (1.05, 6.66)	1.38 (0.61, 3.11)
3 biomarkers low	2.97 (1.15, 7.75)	1.14 (0.28, 4.66)
4 biomarkers low	1.80 (0.31, 10.49)	-

* Low biomarker defined as in the bottom third of the distribution (percentiles defined in Table 2)

[†] Adjusted for risk factors in Model 2: age (continuous), sex, race, region (southeast vs. rest of the U.S.), age*race interaction term, regular aspirin use, systolic blood pressure (continuous), diabetes, current smoking, baseline cardiovascular disease, baseline atrial fibrillation, baseline left ventricular hypertrophy, baseline use of antihypertensive medications, and alcohol use (0, 1-7, and >7 drinks/week).