Received: 1 March 2020 | Accepted: 24 August 2020

DOI: 10.1111/jth.15092

ORIGINAL ARTICLE

Coagulation factor XII, XI, and VIII activity levels and secondary events after first ischemic stroke

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Funding Information
The PROSCIS-B study received funding from the Federal Ministry of Education and Research via the grant Center for Stroke Research Berlin (01 EO 0801).

Abstract

Background: Though risk for recurrent vascular events is high following ischemic stroke, little knowledge about risk factors for secondary events post-stroke exists.

Objectives: Coagulation factors XII, XI, and VIII (FXII, FXI, and FVIII) have been implicated in first thrombotic events, and our aim was to estimate their effects on vascular outcomes within 3 years after first stroke.

Patients/Methods: In the Prospective Cohort with Incident Stroke Berlin (PROSCIS-B) study, we followed participants aged 18 and older for 3 years after first mild to moderate ischemic stroke event or until occurrence of recurrent stroke, myocardial infarction, or all-cause mortality. We compared high coagulation factor activity levels to normal and low levels and also analyzed activities as continuous variables. We used Cox proportional hazards models adjusted for age, sex, and cardiovascular risk factors to estimate hazard ratios (HRs) for the combined endpoint.

Results: In total, 94 events occurred in 576 included participants, resulting in an absolute rate of 6.6 events per 100 person-years. After confounding adjustment, high FVIII activity showed the strongest relationship with the combined endpoint (HR = 2.05, 95% confidence interval [CI] 1.28–3.29). High FXI activity was also associated with a higher hazard (HR = 1.80, 95% CI 1.09–2.98), though high FXII activity was not (HR = 0.86, 95% CI 0.49–1.51). Continuous analyses yielded similar results.

Conclusions: In our study of mild to moderate ischemic stroke patients, high activity levels of FXI and FVIII but not FXII were associated with worse vascular outcomes in the 3-year period after first ischemic stroke.

KEYWORDS

coagulation, factor VIII, factor XI, factor XII, ischemic stroke

Manuscript handled by: Matthew T. Rondina

Final decision: Matthew T. Rondina, 24 August 2020

1 | INTRODUCTION

Globally, stroke remains a leading cause of disability and mortality.[1,2] Following first-ever ischemic stroke, the risk of secondary events is high.[3-5] Although many risk factors for first ischemic stroke have been identified, comparatively little is known about factors that contribute to secondary post-stroke events. In fact, a recent systematic review of biomarkers of hemostasis found no conclusive evidence of a single marker ready for use in practice, largely due to the limited number of existing studies.[6]

The coagulation factors XII, XI, and VIII (FXII, FXI, and FVIII) are promising candidates for further investigation in this context. As the initiating factor in the contact activation system, FXII was quickly implicated in first-ever vascular events in animal studies; however, conflicting evidence exists regarding the potential role of FXII in ischemic stroke events in humans.[7-10] The role of FXI in hypercoagulability has been more consistently demonstrated, and high levels of FXI have been linked to thrombotic events, especially ischemic stroke.[11-13] Given its role in thrombin activation and thrombus formation, it is unsurprising that high levels of FVIII have also been implicated in vascular events.[14,15] FVIII elevation is observed during the acute phase of stroke as part of the inflammatory response;[16] however, a dose-dependent relationship between FVIII and thrombosis, independent of this acute phase response, has also been described.[14,15,17,18] A recent study found that acute ischemic stroke patients with elevated FVIII experienced a higher frequency of recurrent thrombotic events while in hospital.[19] It remains unknown whether this increased risk due to FVIII elevation also persists in the longer term for future incident thrombotic events.

In the present study, we aimed to estimate the effects of FXII, FXI, and FVIII activity levels on risk for secondary vascular events among ischemic stroke patients.

Essentials

- Factors XII, XI, and VIII are linked with first vascular events; role in secondary events unclear.
- We followed adult stroke patients for 3 years or until stroke, myocardial infarction, or death.
- We report confounding-adjusted estimates for effect of factor activities on the combined endpoint.
- High FXI and FVIII but not FXII activities were associated with worse post-stroke vascular outcomes.

2 | MATERIALS AND METHODS

2.1 | Study population

We used data from the Prospective Cohort with Incident Stroke Berlin (PROSCIS-B; clinicaltrials.gov registration number: NCT01363856). This longitudinal, hospital-based, observational cohort study has been described in detail elsewhere.[20] Participants (or legal representatives) provided written informed consent for study participation. The study protocol was approved by the internal review board of the Charité – Universitätsmedizin Berlin (EA1/218/09) and was conducted in accordance with ethical principles described in the Declaration of Helsinki.

In brief, between January 2010 and February 2013, patients aged 18 or older presenting at one of the three tertiary stroke units at the Charité – Universitätsmedizin in Berlin with first-ever stroke (defined by World Health Organization criteria[21]), including ischemic stroke, primary hemorrhage, or sinus venous thrombosis, were recruited. Participants underwent a baseline visit within 1 week of the initial event, including a detailed interview, clinical examination, and the collection of blood samples stored for later analysis. Participants were contacted annually over a period of 3 years via telephone interview to document vital status, any incident cardiovascular events, and to assess functional outcome. Those who were not reachable by phone were mailed surveys.

As shown in Figure 1, in this study, we excluded non-ischemic stroke patients and patients with severe strokes (defined as having a National Institute of Health Stroke Scale [NIHSS] assessment of >15). Overall, activity measurements for at least one coagulation factor were available for 576 PROSCIS-B participants, who were subsequently included in these analyses.

2.2 | Participant characteristics

At baseline, age, sex, and cardiovascular risk factors were assessed. In the baseline clinical assessment, body mass index (BMI, in kg/m^2), high density lipoprotein (HDL, in mg/dL), and low-density lipoprotein (LDL, in mg/dL) cholesterol were measured. Participants were asked to provide information about lifestyle-related risk factors including smoking (never, former, or current); whether they consumed alcohol regularly; and whether they had a history of diabetes mellitus, hypertension, and acute coronary syndrome (myocardial infarction or angina pectoris). The stroke units provided information on whether the patient received recombinant tissue-type plasminogen activator (rt-PA) treatment, the suspected stroke etiology according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification,[22] and the severity of the stroke based on the NIHSS (mild: 0–4, moderate: 5–15, severe: >15).[23]

2.3 | Exposure assessment: coagulation factors

Citrate-buffered blood samples were obtained from PROSCIS-B participants after an overnight fast within 1 week of the initial stroke event and aliquots were stored at −80°C degrees until thawed once for the laboratory assays. Between the initial stroke event and the time of blood sampling, a median of 4 days elapsed (interquartile range [IQR] limits: 3–5). Coagulation factor activity

levels (:C) were measured using a one-stage clotting assay and are reported as percentages of activated normal pooled plasma (standard activity units). Some of the samples had too little plasma; in these cases, FXI:C followed by FXII:C measurements were prioritized as less is known about these factors in the context of secondary vascular risk compared to FVIII:C. Coagulation factor measurements were performed blinded to participant characteristics and outcome status.

2.4 | Outcome: combined endpoint

We generated the combined endpoint outcome as the composite of relevant secondary event occurrence; first of either recurrent stroke, myocardial infarction, or death attributable to any cause during follow-up. During follow-up, participants were requested to provide information about the occurrence of any of these events since the last time of contact. We confirmed these self-reported outcomes using the Charité – Universitätsmedizin hospital discharge records or, when not available, using information obtained from the treating hospital or general practitioner.

We performed additional screening of the Charité hospital records to identify any events of interest not self-reported by participants during follow-up. Information about death from any cause was supplemented using city registration office's records. For one participant, the exact date of death could not be determined and was assigned as the halfway point between last contact and the date on the returned postal questionnaire.

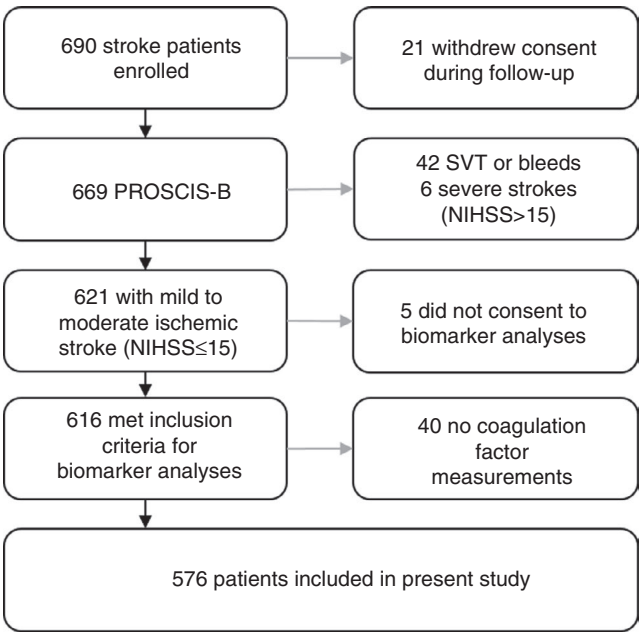

FIGURE 1 Participant inclusion flowchart. Abbreviations: NIHSS, National Institutes of Health Stroke Scale; SVT, sinus venous thrombosis. Of the 40 participants with no recorded coagulation factor activity measurements, 15 had samples that failed in the laboratory and the remainder had no stored citrate available for analysis

2.5 | Endpoint committee

All cardiovascular endpoints were confirmed using medical records from the treating hospital or physician and validated by an independent endpoint committee consisting of two senior vascular neurologists. We used only these committee-confirmed endpoints in our analyses.

2.6 | Statistical analysis

We summarized the baseline characteristics for the full PROSCIS-B cohort using medians and IQR limits for continuous variables and frequencies and percentages for nominal variables.

In the primary analysis, we categorized the FXII:C, FXI:C, and FVIII:C levels into quartile groups and compared participants with the highest fourth (>75th percentile) to the remainder (reference) for each factor. In an additional analysis, for each factor, we analyzed the activity measurements as continuous exposure variables divided by the standard deviation of all measurements of that factor to allow for better comparisons of the estimated effect sizes between the three factors. All reported analyses are complete case analyses.

In the time-to-event analyses, we calculated person-time from the date of the initial ischemic stroke to the date of occurrence of the combined endpoint during follow-up (first occurrence of either recurrent stroke, myocardial infarction, or death by any cause), loss to follow-up, or the study end, whichever came first. We used Kaplan-Meier curves to estimate event-free survivorship and the log-rank test to measure overall crude differences in survivorship curves between groups after visual inspection of fulfillment of the proportional hazards assumption. Dropouts were censored on the date of last contact.

We used Cox proportional hazards models to estimate the hazard ratios (HR) and 95% confidence intervals (CI) for the combined endpoint outcome adjusted for potential confounding factors. We used multiple models for confounding control: Model 1 was adjusted only for age and sex. In Model 2, we additionally adjusted for cardiovascular factors determined to contribute to confounding based on *a priori* knowledge. In addition to age and sex, Model 2 included the continuous variables BMI, HDL, and LDL cholesterol levels; the categorical variable smoking status (never, ever, current); and the following dichotomous variables: regular alcohol consumption, hypertension, diabetes mellitus, and acute coronary syndrome.

As a sensitivity analysis, in a third model (Model 3), we adjusted for all Model 2 covariates as well as rt-PA treatment status and stroke severity (NIHSS). Because these are consequences of the stroke, they could be intermediates in the causal path of interest and may not contribute to confounding directly. However, as they may also be proxies for relevant pre-stroke confounders, we decided to explore how the effect estimates change with their inclusion in Model 3.

We performed all analyses using STATA IC version 14.2 (Stata Corp.). The syntax is available on request from the corresponding author.

3 | RESULTS

Participant characteristics at baseline are displayed in Table 1. PROSCIS participants with mild to moderate ischemic stroke (N = 621) were predominantly male (61%) and had a median age of 69 years (IQR limits: 58–76). Arterial hypertension was observed in 65% and diabetes mellitus in 22% of participants. Twenty percent of participants received rt-PA treatment. Median activity levels for FXII:C, FXI:C, and FVIII:C were 108 (IQR limits: 91–127), 113 (99–130), and 140 (115–166), respectively. At least one of the three coagulation factor measurements was available for 576 participants (93%) who were included in the present analyses. All three factor activity measurements were available for 553 participants. The distribution of high versus low/normal activity levels across the TOAST stroke subtypes is displayed in Table S1 in the Supporting Information. All other variables relevant for this study measured at baseline had <5% missing values aside from the LDL and HDL cholesterol levels, for which 34 participants had missing values.

After a pursuit follow-up time of 3.0 years resulting in 1419.5 contributed person-years, 94 combined endpoint events occurred. Of these, 41 were recurrent ischemic strokes, 5 were myocardial infarctions, and 48 were deaths. The overall crude observed incidence rate for included participants was 6.6 events per 100 person-years. The absolute cumulative risk for the combined outcome during follow-up among the 576 included participants was 16.3%.

We generated Kaplan-Meier curves to compare participants with coagulation factor levels in the highest fourth (>p75) with the remainder for the three factors of interest (Figure 2A-C). In the crude comparison, no significant difference between >p75 and ≤p75 groups of FXII:C was observed in the log-rank test ($P = .48$). However, clear differences were visible for both the FXI:C and FVIII:C curve comparisons. Visually, participants with high levels had consistently higher cumulative probabilities of the combined endpoint compared to the reference group (FXI:C: $P = .06$; FVIII:C: $P = .0001$).

The multivariable adjusted HRs are shown in Table 2. In the fully adjusted model (Model 2), high FXII:C levels (>p75) were not associated with the combined endpoint (HR = 0.86, 95% CI 0.49–1.51). Having high FXI:C levels was associated with a higher hazard for the combined endpoint: (HR = 1.80, 95% CI 1.09–2.98), as was having high FVIII:C levels (HR = 2.05, 95% CI 1.28–3.29), compared to low/normal levels. In the secondary analyses treating the coagulation factor levels as continuous variables, we obtained similar results (Table 2). One standard deviation of FXII:C, FXI:C, and FVIII:C levels corresponded to 29.3, 28.8, and 45.4 units, respectively.

In a sensitivity analysis (Model 3), we further adjusted Model 2 for NIHSS and rt-PA treatment. This additional adjustment did not substantially change the results (Table 2).

TABLE 1 Baseline characteristics of PROSCIS-B participants with mild to moderate ischemic stroke

PROSCIS-B participants with mild to moderate ischemic stroke (N=621)[a]		
Age in years, median (IQRL)	69	(58–76)
Female sex, N (%)	242	(39%)
BMI in kg/m^2, median (IQRL)	27	(24–30)
HDL cholesterol in mg/dL, median (IQRL)	49	(40–60)
LDL cholesterol in mg/dL, median (IQRL)	117	(96–147)
Hypertension, N (%)	406	(65%)
Acute coronary syndrome, N (%)	99	(16%)
Diabetes mellitus, N (%)	137	(22%)
Habitual alcohol consumption, N (%)	217	(35%)
Smoking, N (%)		
Former	201	(33%)
Current	171	(28%)
TOAST subtype, N (%)		
Large-artery atherosclerosis	167	(27%)
Cardioembolism	145	(23%)
Small vessel occlusion	96	(15%)
Other determined etiology	22	(4%)
Undetermined etiology[b]	191	(31%)
NIHSS, N (%)		
Mild, 0–4	470	(76%)
Moderate, 5–15	151	(24%)
Thrombolysis treatment (rt-PA), N (%)	125	(20%)
Coagulation measurements available for at least one factor[c], N (%)	576	(93%)
FXII:C[d], median (IQRL)	108	(91–127)
FXI:C, median (IQRL)	133	(99–130)
FVIII:C, median (IQRL)	140	(115–166)

Abbreviations: BMI, body mass index; FVIII:C, coagulation factor VIII activity; FXI:C, coagulation factor XI activity; FXII:C, coagulation factor XII activity; HDL, high-density lipoprotein; IQRL, interquartile range limits; LDL, low-density lipoprotein; NIHSS, National Institutes of Health Stroke Scale; PROSCIS-B, Prospective Cohort with Incident Stroke Berlin; rt-PA, recombinant tissue-type plasminogen activator; TOAST, stroke etiology according to Trial of Org 10172 in Acute Stroke Treatment.

[a]Owing to missing data, percentages may not total 100%. 5 participants did not consent to biomarker measurements and were excluded. All variables have <5% missing values except for the coagulation factor and cholesterol measurements. In total, 34 study participants were missing LDL and HDL cholesterol measurements, and 40 participants were missing all three coagulation factor activity measurements.

[b]Includes cryptogenic stroke despite complete work-up, stroke of two or more etiologies, and stroke with incomplete work-up.

[c]Laboratory measurements available for at least one of the coagulation factors of interest; 553 had all three coagulation factor activity measurements.

[d]Activities were measured as percentages of activated normal pooled plasma.

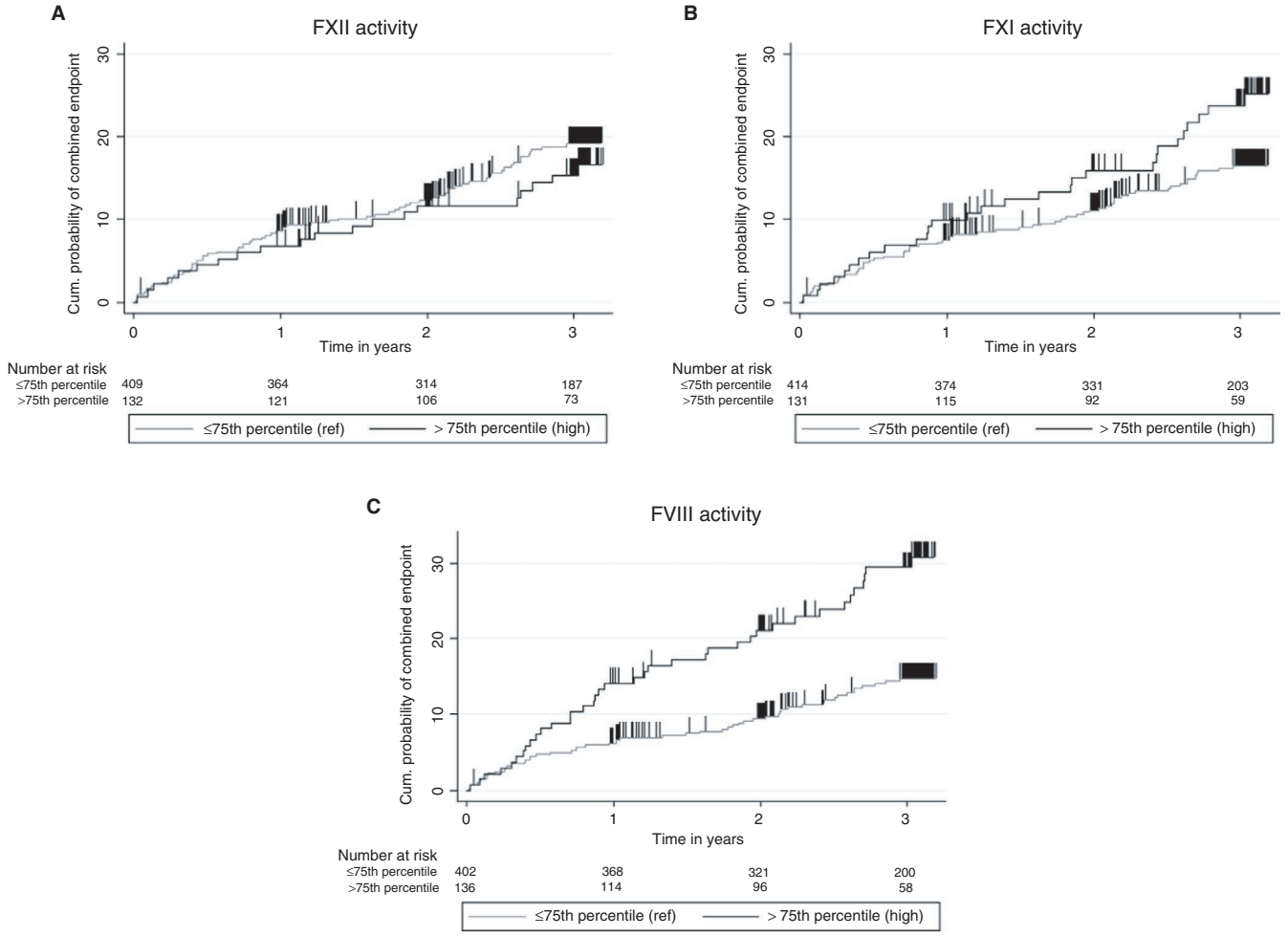

FIGURE 2 Cumulative probabilities of the combined vascular endpoint. Kaplan-Meier estimates among stroke patient participants with high activity levels (>75th percentile) of each indicated coagulation factor (Panel A) FXII, (Panel B) FXI, and (Panel C) FVIII, compared to those with lower activity levels of each factor (≤75th percentile, reference). Activity levels were measured as percentages of activated normal pooled plasma. Abbreviations: Cum., cumulative; FVIII, coagulation factor VIII; FXI, coagulation factor XI; FXII, coagulation factor XII; ref, reference.

4 | DISCUSSION

In this prospective patient cohort study of individuals with mild to moderate ischemic stroke, having high levels of FXI or FVIII activity was associated with a higher hazard for the combined vascular endpoint within 3 years compared with individuals having low/normal levels after confounding adjustment. Such a relationship was not observed for having high FXII activity. Our findings expand on the recent literature and fill some important gaps with longitudinal results.

Specifically in the context of post-stroke outcomes, the search for meaningful etiologic or prognostic hemostatic biomarkers has not been straightforward. A recent systematic review of hemostatic biomarkers in ischemic stroke revealed a large heterogeneity in existing studies and did not find enough evidence to provide clear recommendations for a prognostic marker to be used in practice; however, some biomarkers, including FXI and FVIII, seemed promising in some of the included studies.[6]

In general, the existing evidence on coagulation factor activity and post-stroke outcomes is quite limited. Our findings pertaining to FXI activity and long-term outcomes add to previous findings from a cross-sectional study of ischemic stroke and transient ischemic attack patients aged 70 or younger, which concluded that the presence of circulating activated FXI during the acute phase of cerebral ischemia (defined as having a detectable response to inhibitory monoclonal antibodies in plasma harvested within 72 hours of symptom onset) at hospital admission was associated with a higher NIHSS score, higher Modified Rankin Scale (mRS), and lower Barthel index at discharge.[24]

Another study found that acute ischemic stroke patients with elevated FVIII (>1.50 IU/mL) at hospital admission experienced a higher frequency of recurrent thrombotic events (defined as new ischemic stroke, progressive stroke, myocardial infarction, deep vein thrombosis, or pulmonary embolism) while in hospital.[19] Our findings add to these results by showing that this relationship also appears to persist in the longer term for future incident events. Regarding ischemic stroke patients who underwent thrombolysis, a study found that having elevated FVIII activity levels (defined as >168%, the upper limit of the reference level), both immediately and 24 hours

after thrombolysis, led to a higher risk for poor functional outcome (mRS ≥ 3) at 90 days.[25]

There have been some important recent developments regarding hemostatic factors in the context of first stroke that may also provide relevant insights in the context of secondary prevention. For instance, FXI appears to be a stronger risk factor for first ischemic stroke than myocardial infarction[26] and may therefore be a particularly attractive target. Numerous laboratory and animal studies have further demonstrated a prothrombotic role of FXII and FXI in thrombosis but non-integral role of these factors in normal hemostasis, making them promising targets for future anticoagulant drug development with potentially lower bleeding risk.[7,27,28] In addition to the successful trial conducted among knee arthroplasty patients, in which FXI antisense oligonucleotides prevented venous thrombotic events without increasing bleeding risk,[29] a recent genetic study investigating variants known to alter FXI levels and increase relative activated partial thromboplastin time found that genetic disposition to lower FXI levels was associated with lower odds for ischemic stroke without increasing risk for major bleeding.[30] This decrease was equivalent to the FXI level reduction that can be achieved through pharmacological modulation.[30]

Furthermore, a 2018 study that used two-sample Mendelian randomization found that genetically determined FXI levels had a causal effect on the risk of any ischemic stroke but not myocardial infarction or intracerebral hemorrhage, with the strongest effect observed amongst the cardioembolism subgroup.[31] Shortly after, a 2019 study that also used Mendelian randomization techniques in a larger meta-analysis, integrating phenomic, genomic, and proteomic databases, assessed the role of 653 proteins as potential mediators for ischemic stroke subtypes and relevant side effects.[32] In this study, genetically determined FXI levels were identified as one of five causal mediators of ischemic stroke, with the cardioembolic subtype appearing to drive this effect.[32] In both studies, no adverse side effects appeared to be linked to the genetic influences on variation in FXI levels, providing further justification for clinical trials on FXI-related interventions in the context of ischemic stroke.[32] Another study published in 2020 used two-sample Mendelian randomization to assess the causal relationships among numerous coagulation factor and other hematological traits on ischemic stroke and its subtypes using data from the MEGASTROKE Consortium.[33] Specifically, genetically higher levels of FXI activity and FVIII antigen were each found to be associated with increased ischemic stroke risk as well as specific risk for the cardioembolic subtype, but not with small-vessel stroke risk.[33] Interestingly, reduced FVIII activity was associated with ischemic stroke risk, and specifically the cardioembolic and large artery atherosclerosis subtypes (the latter only among the European population).[33] Our study was not powered to investigate stratum-specific effects across the stroke subtypes, but this area warrants future research also in the context of secondary event prevention.

In light of our findings that stroke patients with high FXI:C had a higher risk for secondary events after first stroke, the population of stroke patients with high FXI levels may particularly benefit from such targeted interventions. FXI:C may be a promising biomarker for identifying individuals who are most likely to benefit from such interventions.

TABLE 2 Hazard ratios from Cox proportional hazards regression models for FXII:C, FXI:C, and FVIII:C

FXII:C[a]	n	Combined EP events	HR1[b]	95% CI	HR2[c]	95% CI	HR3[d]	95% CI
≤p75	428	71	1	ref	1	ref	1	ref
>p75 (high)	137	20	1.00	(0.60–1.67)	0.86	(0.49–1.51)	0.91	(0.51–1.62)
Per SD[e]	565	91	0.94	(0.76–1.16)	0.90	(0.71–1.13)	0.92	(0.73–1.17)
FXI:C	**n**	**Combined EP events**	**HR1**	**95% CI**	**HR2**	**95% CI**	**HR3**	**95% CI**
≤p75	433	63	1	ref	1	ref	1	ref
>p75 (high)	137	29	1.81	(1.15–2.84)	1.80	(1.09–2.98)	1.84	(1.11–3.07)
Per SD	570	92	1.26	(1.04–1.53)	1.26	(1.00–1.58)	1.23	(0.98–1.55)
FVIII:C	**n**	**Combined EP events**	**HR1**	**95% CI**	**HR2**	**95% CI**	**HR3**	**95% CI**
≤p75	420	54	1	ref	1	ref	1	ref
>p75 (high)	140	38	2.10	(1.38–3.19)	2.05	(1.28–3.29)	2.18	(1.35–3.52)
Per SD	560	92	1.33	(1.11–1.59)	1.37	(1.10–1.71)	1.37	(1.10–1.72)

Abbreviations: CI, confidence interval; EP, endpoint; FVIII:C, coagulation factor VIII activity; FXI:C, coagulation factor XI activity; FXII:C, coagulation factor XII activity; HR, hazard ratio; p75, 75th percentile; ref, reference; SD, standard deviation.

[a]Activities were measured as percentages of activated normal pooled plasma.

[b]Model 1: Adjusted for age and sex.

[c]Model 2: Adjusted for Model 1 variables plus BMI, HDL, LDL, smoking status, regular alcohol consumption, hypertension, diabetes mellitus, and acute coronary syndrome (see Methods for detailed variable definitions).

[d]Model 3 (sensitivity analysis): Adjusted for Model 2 variables plus thrombolysis treatment (rt-PA) status and NIHSS (National Institutes of Health Stroke Scale).

[e]One standard deviation of FXII:C, FXI:C, and FVIII:C levels were 29.3, 28.8, and 45.4 units, respectively.

4.1 | Strengths and limitations

Though the relationships between coagulation factors FXII, FXI, and FVIII and primary thrombotic event risk, for both venous and arterial events, have been well studied, to the best of our knowledge, this is the first study to investigate their role in long-term vascular event risk after first stroke. Furthermore, a limited number of studies have included multiple coagulation factors in a single study, and existing studies often lack follow-up beyond hospital discharge. Coagulation activity was assayed using state-of-the-art machinery on fresh-frozen, once-thawed plasma samples as opposed to antigen level measurements. Our longitudinal design with 94 observed outcome events also afforded us the opportunity to contribute our analyses of long-term outcome risk to the literature, which is currently limited to cross-sectional and very-short-term designs.

We were able to screen for additional, unreported clinical endpoints of interest in the Charité University Hospital medical records to supplement the information provided by the patients; however, unreported clinical endpoints presenting at other clinics in Berlin or elsewhere may have been missed. When possible, we made an effort to validate any patient-reported events by requesting forwarding of medical records from other hospitals and clinics. We further confirmed the vital status for all participants at the end of the study via the local citizen's registration office in Berlin; however, due to legal restrictions, we could not obtain specific cause information from the death certificates.

Some limitations should also be considered when interpreting our results. First, self-reported patient characteristics, such as the lifestyle-related factors and the presence of chronic diseases at baseline, may be prone to recall bias. A set of standard operating procedures and training was provided for the study nurses in an effort to improve consistency in the measurements made at study enrollment. Although we believe that we have included the most important potential sources of confounding in the adjusted models, we cannot rule out that some residual confounding may be present due to unmeasured factors.

Second, we emphasize that the coagulation factor activity levels were measured in blood samples that were taken after the index stroke event, and the initial stroke event itself may activate the coagulation system.[34] Though FXII and FXI are not known to change dramatically during the acute phase of stroke, this phenomenon has been well documented for FVIII. This means that our findings for FVIII are likely a mixing of elevated FVIII as part of the acute phase and high pre-stroke FVIII levels (increase of thrombotic event risk independent of the acute phase[18]). It is also possible that these activity levels changed within the first week post-stroke, during which the blood was drawn. About one fifth of the study participants received rt-PA treatment, and we cannot rule out that some may have been on anticoagulation therapy at the time of the initial stroke event. Time-standardized measurements should be considered in future confirmatory studies, and sequential measurements could provide additional relevant insights into the changes that occur shortly after stroke.

Our reported results apply to a cohort comprised of first-ever mild to moderate ischemic stroke patients. The six patients enrolled with a baseline NIHSS >15 in the PROSCIS-B study were excluded to limit the heterogeneity of the cohort. Readers should take care not to extrapolate our conclusions to severe patients (NIHSS >15) or patients with severe comorbidities or complications (such as sepsis) who may have been less likely to participate in our study.

We do not expect that the censoring of individuals lost to follow-up was differential with respect to exposure status. However, it is feasible, despite our efforts to confirm unreported endpoints, that those who were lost to follow-up may have been more likely to experience one of the combined vascular endpoints compared to the participants actively remaining in the study. Lastly, due to a limited number of endpoint events per included independent variable in Models 2 and 3, we acknowledge that the measured associations of interest in these models may be imprecise and could even be biased in the direction of more extreme values. However, especially for observational studies with causal questions like ours in which full confounding control is crucial, simulation results indicate that use of less rigid events per variable criteria is often justifiable for Cox regression analysis in terms of important model performance measures and in the range of 5 to 9 events per variable (our Models 2 and 3), such problems are uncommon.[35]

5 | CONCLUSIONS

Our study of mild to moderate ischemic stroke patients indicates that high levels of FXI:C or FVIII:C measured within 1 week of the index event may contribute to unfavorable vascular outcomes after stroke in the longer term (3 years). We did not observe a clear relationship with FXII:C. Further research in this area should focus on obtaining time-standardized and repeated measures of coagulation factor activities after stroke. In the context of secondary prevention, we demonstrated that individuals with high levels of FXI:C after stroke have an increased risk for secondary events. This knowledge may be beneficial for potential future treatment strategies involving drugs targeting FXI.

ACKNOWLEDGMENTS

We thank P. Noordijk, L. Mahic, and A. Hoenderdos from the Leiden University Medical Center for performing laboratory assays and J. Thümmler for PROSCIS-B data management. We are indebted to the participants of the PROSCIS-B for their commitment. JLR is a PhD candidate at the Charité – Universitätsmedizin Berlin, and this work is submitted in partial fulfillment of her PhD requirements. Open access funding enabled and organized by ProjektDEAL.

CONFLICTS OF INTEREST

Jessica L. Rohmann, Sophie K. Piper, Thomas G. Liman, and Bob Siegerink report no disclosures related to this work. Shufan Huo reports funding from the Sonnenfeld Foundation from January 2017 until March 2018. Pia S. Sperber reports funding from FAZIT-STIFTUNG between March 2018 and March 2020. Frits R.

Rosendaal is listed on patents of several prothrombotic variants, including factor XI. Peter U. Heuschmann reports research grants from the German Ministry of Research and Education, German Research Foundation, European Union, Charité, Berlin Chamber of Physicians, German Parkinson Society, University Hospital Würzburg, Robert-Koch-Institute, German Heart Foundation, Federal Joint Committee (G-BA) within the Innovationsfond, Charité – Universitätsmedizin Berlin (within MonDAFIS; supported by an unrestricted research grant to the Charité from Bayer), University Göttingen (within FIND-AF-randomized; supported by an unrestricted research grant to the University Göttingen from Boehringer-Ingelheim), and University Hospital Heidelberg (within RASUNOA-prime; supported by an unrestricted research grant to the University Hospital Heidelberg from Bayer, BMS, Boehringer-Ingelheim, Daiichi Sankyo), outside of the submitted work. Matthias Endres reports grant support from Bayer, the German Research Foundation (DFG), the German Federal Ministry of Education and Research (BMBF), the German Center for Neurodegenerative Diseases (DZNE), the German Centre for Cardiovascular Research (DZHK), the European Union, Corona Foundation, and Fondation Leducq; fees paid to the Charité from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb/Pfizer, Daiichi Sankyo, Amgen, GlaxoSmithKline, Sanofi, Covidien, and Novartis, all outside of the submitted work.

AUTHOR CONTRIBUTIONS

J. L. Rohmann contributed to study conception and design, coagulation factor assays, analysis and interpretation of the data, manuscript drafting, and critical revision. S. Huo and P. S. Sperber contributed to data collection, interpretation, and critical revision of the manuscript. S. K. Piper contributed to project data management and critical revision of the manuscript. F. R. Rosendaal facilitated the laboratory assays and their interpretation and contributed to critical revision of the manuscript. P. U. Heuschmann and M. Endres were involved in original study conception and design as well as critical revision of the manuscript. T. G. Liman also contributed to study conception and design, was responsible for data collection, and contributed to critical revision of the manuscript. B. Siegerink contributed to study concept and design, analysis and interpretation of data, critical revision of the manuscript, and provided project supervision.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Rohmann JL, Huo S, Sperber PS, et al. Coagulation factor XII, XI, and VIII activity levels and secondary events after first ischemic stroke. *J. Thromb. Haemost*. 2020;18:3316–3324. https://doi.org/10.1111/jth.15092