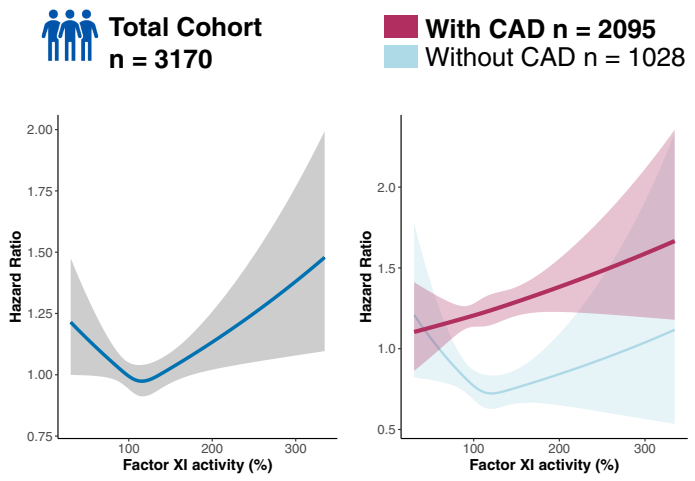
Med

Report

# Non-linear association of coagulation factor XI with mortality

## Graphical abstract




**A** FXI activity shows a U-shaped relationship with overall mortality

**B** Coronary artery disease (CAD) modulates the association

## Highlights

- Both low and high FXI activity levels were linked to increased mortality risk
- In coronary artery disease, FXI activity was linearly associated with mortality
- NT-proBNP modified the FXI-activity-dependent risk of mortality
- Findings suggest a need for personalized FXI-targeted treatment strategies

## Authors

Katsiaryna Prystupa, Martin Heni, Sebastian Hörber, ..., Michael Roden, Winfried März, Robert Wagner

## Correspondence

winfried.maerz@synlab.com

## In brief

This study shows a non-linear association between coagulation factor XI activity and mortality in individuals undergoing coronary angiography. Both low and high FXI activity levels were associated with increased risk, but the pattern differed according to coronary artery disease and heart failure, underscoring the need for personalized approaches when targeting this pathway.

**Translation to Patients**

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Report

# Non-linear association of coagulation factor XI with mortality

Katsiaryna Prystupa,[1,2,16] Martin Heni,[3,4,16] Sebastian Hörber,[2,4,5] Andreas Peter,[2,4,5] Graciela Delgado,[6,7] Marcus Kleber,[6,8] Peter Hellstern,[9] Malte Kelm,[10] Hajime Yamazaki,[11,12] Michael Roden,[1,2,13] Winfried März,[6,14,15,16,17,*] and Robert Wagner[1,2,13,16]

[1]Institute for Clinical Diabetology, German Diabetes Center (DDZ), Leibniz Center for Diabetes Research at Heinrich-Heine University, Düsseldorf, Germany
[2]German Center for Diabetes Research (DZD), Partner Düsseldorf, Neuherberg, Germany
[3]Division of Endocrinology and Diabetology, Internal Medicine 1, University Hospital Ulm, Ulm, Germany
[4]Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital Tübingen, Tübingen, Germany
[5]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen, Tübingen, Germany
[6]Vth Department of Medicine (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
[7]Center for Preventive Medicine and Digital Health Baden-Württemberg (CPDBW), Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[8]SYNLAB MVZ für Humangenetik Mannheim GmbH, Mannheim, Germany
[9]Center of Hemostasis and Thrombosis, Zurich, Switzerland
[10]Department of Cardiology, Pulmonology and Vascular Medicine, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany
[11]Section of Clinical Epidemiology, Department of Community Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[12]Center for Innovative Research for Communities and Clinical Excellence (CiRC2LE), Fukushima Medical University, Fukushima, Japan
[13]Department of Endocrinology and Diabetology, Medical Faculty and University Hospital, Heinrich Heine University, Düsseldorf, Germany
[14]SYNLAB Academy, SYNLAB Holding Deutschland GmbH, Augsburg and Mannheim, Germany
[15]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
[16]These authors contributed equally
[17]Lead contact
*Correspondence: winfried.maerz@synlab.com
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**CONTEXT AND SIGNIFICANCE** Factor XI (FXI) is being investigated as a novel target for anticoagulant therapies. Its broader physiological role, however, is still not fully clear. This study assessed the association between FXI activity and mortality in a cohort at increased risk of cardiac death. Both low and high levels of FXI were linked to increased mortality. The shape of this relationship changed by coronary artery disease (CAD) status and by heart failure biomarker N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels: a U-shaped pattern was observed in individuals without CAD, whereas in those with CAD, mortality increased linearly with FXI activity. These results suggest a complex and context-dependent role for FXI, highlighting the importance of personalized therapeutic strategies when considering targeting FXI.

## SUMMARY

**Background:** Coagulation factor XI (FXI) influences both thrombotic risk and myocardial function, making its relationship with mortality crucial for guiding therapies, especially in coronary artery disease (CAD).
**Methods:** We analyzed data from 3,170 participants who underwent coronary angiography; 67% were diagnosed with CAD. Participants were followed for a median of 14.5 years. Mortality risk was assessed using Cox proportional hazards models with restricted cubic splines and Wald statistics. Models were adjusted for age, sex, BMI, and further cardiovascular risk factors. Interactions between FXI activity, N-terminal pro-B-type natriuretic peptide (NT-proBNP), and CAD were explored.
**Findings:** A U-shaped association between FXI activity and mortality was observed ($p$ = 0.027), with the lowest risk at an FXI activity of 115.6%. Among patients without CAD, this U-shaped relationship persisted. In contrast, patients with CAD demonstrated a linear relationship, where higher FXI activity correlated with

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increased mortality ($p$ interaction < 0.0001). NT-proBNP levels significantly modified these associations, particularly in patients with CAD.

**Conclusions:** These findings emphasize the dual role of FXI activity in hemostasis, which could have profound implications for pharmacological interventions. The variable effects of FXI activity based on underlying cardiovascular conditions suggest that a personalized approach to treatment is necessary. Consequently, future studies on FXI inhibitors should carefully examine these modulating factors to optimize therapeutic strategies.

**Funding:** The LURIC study was supported by the Ludwigshafen Heart Centre and academic collaborators, including the universities of Freiburg, Ulm, and Düsseldorf and the Centre Nationale de Genotypage in France, through internal institutional resources.

## INTRODUCTION

Recently, Cao et al. described a novel liver-heart axis mediated by coagulation factor XI (FXI). In animal models, liver-derived FXI reduces inflammation, fibrosis, and cardiac diastolic dysfunction through activation of the bone morphogenetic protein (BMP)-SMAD1/5 pathway in cardiomyocytes.[1] In humans, FXI has been inversely correlated with the E/e' ratio, an echocardiographic indicator of diastolic heart failure.[1] While these data suggest a protective effect of coagulation FXI against fibrotic remodeling, previous evidence also indicates its involvement as a risk factor of cardiovascular disease.[2] To investigate these pleiotropic effects of FXI on human health, we analyzed the association of FXI with mortality in a cohort at increased risk of cardiac death.

## RESULTS

### Non-linear association between FXI activity and mortality

In the entire cohort, a U-shaped relationship between FXI activity and mortality was observed, as evidenced by the significant non-linear term in the Cox model ($p$ = 0.027, Figure 1A), independent of age, sex, and body mass index (BMI). The lowest mortality risk was noted at an FXI activity of 115.6%, corresponding to a hazard ratio (HR) of 0.97, with the baseline HR of 1 at the FXI activity equal to 98%. Individuals with an FXI activity of less than 60% exhibited an HR higher than 1.12. Conversely, those with FXI activity levels exceeding 150% demonstrated HRs above 1.03.

To account for additional clinically relevant cardiovascular risk factors, we next extended our multivariable model to include non-high-density-lipoprotein (HDL) cholesterol, estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation, and mean arterial pressure. These variables were available in 3,165 participants. Even with these additional adjustments, the non-linear association between FXI activity and all-cause mortality remained statistically significant ($p$ = 0.0366, Figure S2).

Of note, there was a linear association between FXI activity and low-density lipoprotein (LDL) cholesterol levels ($p$ < 0.001), consistent with previous reports.[3,4] Sex-based effect modification was not observed ($p_{interaction(FXI*sex)}$ = 0.31).

### FXI activity and cardiovascular mortality

To evaluate whether the observed association with mortality was driven by cardiovascular causes, we performed a sensitivity analysis restricted to adjudicated major adverse cardiac event (MACE)-related mortality (including myocardial infarction, stroke, and cardiovascular death). In this analysis ($n$ = 3,170, events = 590), the non-linear association between FXI activity and mortality remained statistically significant ($p$ = 0.041, Figure S3), consistent with our findings for total mortality.

### Effect modification by NT-proBNP and coronary artery disease

N-terminal pro-B-type natriuretic peptide (NT-proBNP), a key biomarker of heart failure,[5] was measured in 3,123 participants at baseline in the LURIC study. Our analysis also revealed a significant U-shaped association between FXI activity and NT-proBNP levels, where extremes in FXI activity correlate with increased NT-proBNP ($p$ = 0.0004).

In the interaction model using restricted cubic splines for FXI activity, both NT-proBNP and coronary artery disease (CAD) modulated the effect of FXI activity on mortality ($p_{interaction(FXI*NT\text{-}proBNP*CAD)}$ < 0.0001). Independent of NT-proBNP, sex, age, and BMI, in patients with CAD, higher FXI activity was associated with mortality in a linear manner (Figure 1B). In contrast, in patients without CAD, there was a U-shaped relationship, similar to the one in the unstratified cohort (Figure 1B). When heart failure severity was stratified according to tertiles of NT-proBNP in patients without (Figure 1C) and with (Figure 1D) CAD, we observed different associations of FXI activity levels and mortality. NT-proBNP tertiles significantly influenced the association between FXI activity and mortality in patients with CAD. Specifically, moderate elevation in FXI activity was linked to lower mortality only in those with the highest NT-proBNP, while the opposite effect was observed in patients with the lowest NT-proBNP (Figure 1D).

## DISCUSSION

FXI activity shows a non-linear association with mortality, with patterns strongly dependent on the presence of CAD and heart failure. Moderately elevated levels may play a beneficial role independent of heart failure, but this appears to be restricted to patients without CAD. This finding aligns with FXI's protective role on cardiomyocytes via the anti-fibrotic/anti-inflammatory BMP-SMAD1/5 pathway.[1] In contrast, in those with CAD, the promotion of plasmatic coagulation by FXI may dominate, aggravating other mechanisms leading to mortality, thereby offsetting FXI's protective effects in cardiac remodeling. In this

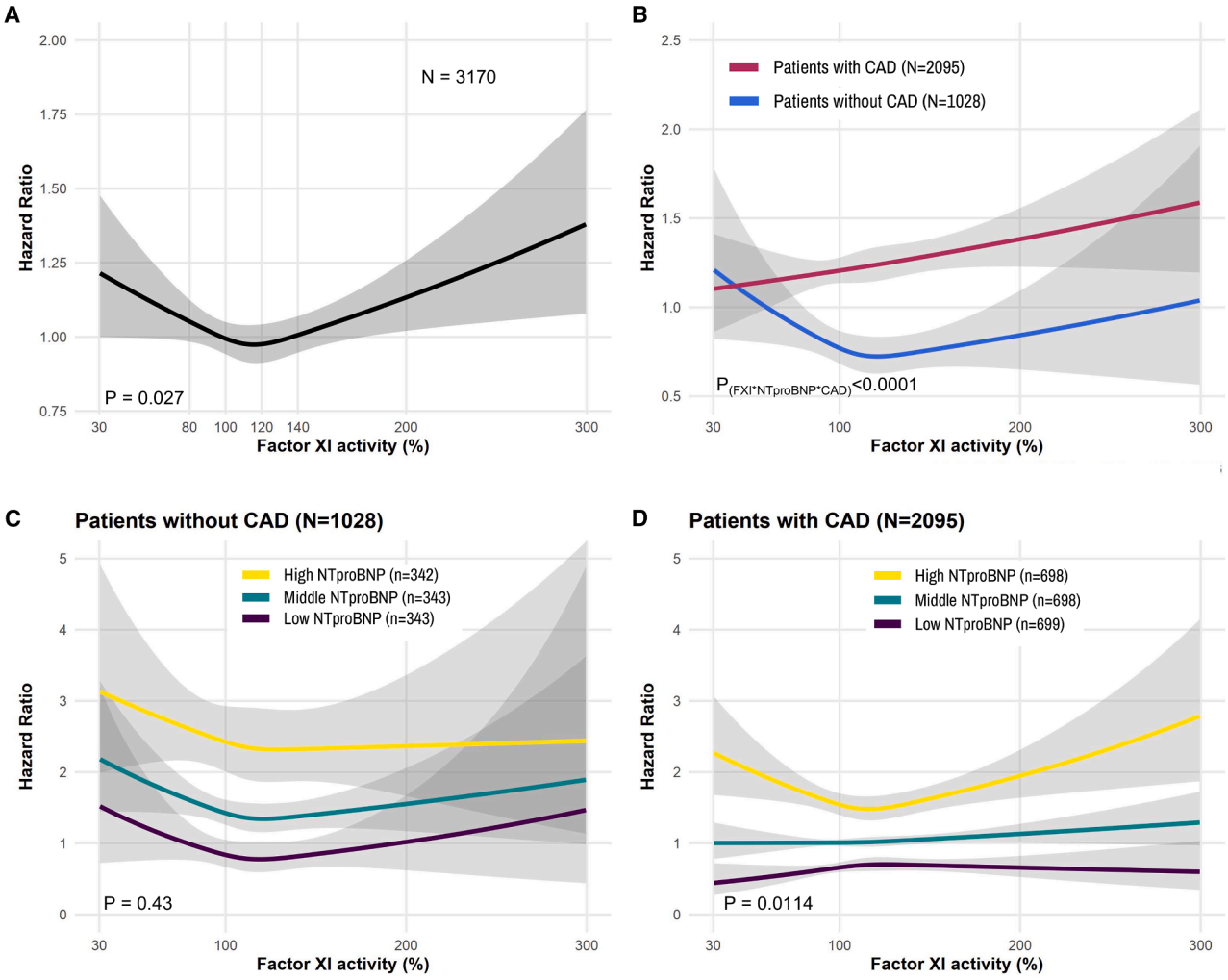


**Figure 1. Non-linear association of factor XI activity with mortality: Interaction with CAD and NT-proBNP**

(A) In the entire cohort, there is a U-shaped relationship between factor XI activity and mortality.

(B) Factor XI activity has different association with mortality dependent on coronary artery disease (CAD).

(C and D) Factor XI activity's association is differently modulated by NT-proBNP tertiles in patients without CAD (C) and with CAD (D).

(B–D) NT-proBNP complete-case subset (*N* = 3,123).

Statistical models are adjusted for sex, age, BMI. In the model displayed in (B), an additional interaction with NT-proBNP was used. Shaded areas represent 95% confidence intervals.

setting, a protective role of FXI is only present in advanced heart failure, where protection against remodeling may outweigh procoagulant properties. Our data support that FXI has multiple and context-dependent roles in human physiology. Consequently, pharmacological inhibition of FXI in heart failure, CAD, or venous thromboembolism may have a narrow therapeutic window, with effects likely to vary by underlying disease, and therefore, these modulating factors should be explicitly investigated in future studies testing FXI inhibitors.

To ensure that these associations were not explained by classical cardiovascular risk factors, we additionally adjusted our analyses for non-HDL cholesterol, eGFR, and blood pressure. Previous studies have reported correlations between FXI and lipid levels,[3,4] effects of statin therapy on FXI,[3] and increased mortality at low FXI levels in heart failure cohorts.[6] Recent studies have further explored the relationship between FXI and mortality. Yap et al. described an association of FX and FXI with cardiac but not all-cause mortality,[7] while Paszek et al. reported a predictive role of FXI for mortality in a small cohort of patients with diabetes.[8] Although these studies provided important insights, they primarily employed linear models and were conducted in different population settings with limited covariate adjustment. Our results extend these earlier observations by using non-linear modeling with multivariable adjustment in a large cohort with coronary angiography and long-term mortality follow-up, revealing that the association of FXI activity with mortality is non-linear, independent of multiple other risk factors, but modified by NT-proBNP and CAD status.

**Table 1. Baseline characteristics of the cohort (*n* = 3,170)**

| Variable | Value | Missing, *n* |
|---|---|---|
| Age, years | 63.6 (56.3, 70.6) | 0 |
| Sex — female, *n* (%) | 976 (30.8) | 0 |
| Sex — male, *n* (%) | 2,194 (69.2) | 0 |
| Race, *n* | 3,106 | 63 |
| Race — White, *n* (%) | 3,106 (100) | – |
| Race — Asian, *n* (%) | 1 (<0.1) | – |
| Race — African, *n* (%) | 0 (0.0) | – |
| BMI, kg/m$^2$ | 27.06 (24.7, 29.7) | 0 |
| CAD (angiographic ≥ 50% stenosis), *n* (%) | 2,132 (67.3) | 0 |
| Smoking status | | 0 |
| Never, *n* (%) | 1,149 (36.2) | – |
| Former, *n* (%) | 1,402 (44.2) | – |
| Active, *n* (%) | 619 (19.5) | – |
| Diabetes (ADA), *n* (%) | 1014 (32.0) | 0 |
| eGFR (CKD-EPI cystatin C), mL/min/1.73 m$^2$ | 80.8 (65.9, 94.6) | 4 |
| High-sensitive C-reactive protein, mg/L | 3.4 (1.3, 8.6) | 4 |
| LDL cholesterol, mg/dL | 114.0 (94.0, 138.0) | 1 |
| HDL cholesterol, mg/dL | 37.0 (31.0, 45.0) | 1 |
| Non-HDL cholesterol, mg/dL | 150.0 (127.0, 177.0) | 1 |
| Triglycerides, mg/dL | 146.0 (109.0, 201.0) | 1 |
| Lipoprotein(a), mg/dL | 16.0 (7.3, 38.0) | 3 |
| Glycosylated hemoglobin (HbA1c), % | 6.0 (5.6, 6.6) | 4 |
| Systolic blood pressure, mmHg | 140.3 (123.5, 156.7) | 0 |
| Diastolic blood pressure, mmHg | 80.7 (73.0, 88.3) | 0 |
| Mean arterial pressure, mmHg | 110.7 (101.1, 121.0) | 0 |
| FXI activity, % of normal plasma | 111.7 (89.5, 139.1) | 0 |
| NT-proBNP, ng/L | 294.0 (106.0, 875.5) | 47 |
| Aspirin/other antiplatelet = yes (%) | 2275 (71.8) | 0 |
| Vitamin K antagonis = yes (%) | 206 (6.5) | 0 |
| CSE inhibitor (statin) = yes (%) | 1498 (47.3) | 0 |
| Follow-up time, years | 14.5 (8.5, 17.5) | 0 |

Continuous variables are represented as median (interquartile range). BMI, body mass index; CAD, angiography-confirmed coronary artery disease (≥50% stenosis); ADA, American Diabetes Association; eGFR, estimated glomerular filtration rate (CKD-EPI cystatin C); NT-proBNP, N-terminal pro-B-type natriuretic peptide; FXI, factor XI activity (percentage of normal plasma).

## Limitations of the study

The LURIC cohort consists exclusively of individuals of German ancestry, and all participants underwent coronary angiography at baseline. As a result, the cohort is enriched for individuals with suspected or established cardiovascular disease, and findings may not be generalizable to broader or more ethnically diverse populations. Furthermore, cause-of-death adjudication was not available for all participants, limiting the ability to conduct comprehensive cause-specific mortality analyses. Nevertheless, in a sensitivity analysis restricted to adjudicated MACE-related deaths, the association between FXI activity and mortality remained significant, supporting the cardiovascular relevance of our findings. Although we adjusted for major cardiovascular risk factors, the precise biological mechanisms linking FXI activity to mortality are not yet fully elucidated. Experimental and translational studies suggest possible pleiotropic effects of FXI on vascular integrity,[9] inflammation,[10] and blood pressure regulation,[11] which warrant further investigation.

## Conclusions

Together, these findings highlight the context-dependent effects of FXI on mortality and underscore the importance of considering underlying cardiovascular status when evaluating FXI as a therapeutic target. Further mechanistic and clinical studies will be essential to delineate the balance between protective and prothrombotic effects in different patient populations.

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Prof. Winfried März (winfried.maerz@synlab.com; winfried.maerz@luric-online.de).

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- Due to the limitations of the original ethical approval and patient consent and the articles of the LURIC Study GmbH, participant data cannot be made publicly available. De-identified participant data underlying the findings will be made available within ethics approval constraints and following a data use agreement or in the context of an approved scientific collaboration.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## ACKNOWLEDGMENTS

We thank the LURIC research team for their involvement in patient recruitment and processing of specimens and data and the employees of the laboratories of the General Hospital Ludwigshafen and the Universities of Freiburg, Ulm, and Graz. The LURIC study was supported by the Ludwigshafen Heart Centre and academic collaborators, including the universities of Freiburg, Ulm, and Düsseldorf and the Centre Nationale de Genotypage in France, through internal institutional resources.

## AUTHOR CONTRIBUTIONS

Formal analysis, K.P.; writing – original draft, K.P. and M.H.; writing – review & editing, S.H., A.P., G.D., M. Kleber, P.H., M. Kelm, H.Y., M.R., W.M., and R.W.; conceptualization, M.H., W.M., and R.W.; supervision, M.H.; statistical analysis, K.P. and R.W.; data curation, G.D., M. Kleber, and W.M.; methodology, P.H. and W.M.; formal analysis, H.Y. and R.W. R.W., M.H., K.P., and W.M. had unrestricted access to all data. All authors agreed to submit the

manuscript, read and approved the final draft, and take full responsibility of its content, including the accuracy of the data and statistical analysis.

## DECLARATION OF INTERESTS

Outside of the current work, K.P. reported a lecture fee from Berlin-Chemie AG. M.H. reports participation on an advisory board for Boehringer Ingelheim, Chiesi/Amryt, and Sanofi and lecture fees/travel support from Chiesi/Amryt, AstraZeneca, Boehringer Ingelheim, Lilly, Novartis, Novo Nordisk, and Sanofi. R.W. served on an advisory board for Akcea Therapeutics, Daiichi Sankyo, Sanofi, Eli Lilly, and Novo Nordisk. M. Kleber and W.M. report employment by SYNLAB Holding Deutschland GmbH. M.R. received fees as a member of advisory boards or as a speaker from Allergan, Boehringer Ingelheim Pharma, Bristol-Myers Squibb, Eli Lilly, Fishawack Group, Gilead Sciences, Novartis Pharma, Intercept Pharma, Inventiva, Novo Nordisk, Target RWE, and Terra Firma and has been involved with clinical trial research for Boehringer Ingelheim, DanoneNutricia Research, and Sanofi-Aventis, all outside of the submitted work.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
- QUANTIFICATION AND STATISTICAL ANALYSIS

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2025.100934.

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# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Biological samples | | |
| Human plasma/serum samples (LURIC Prospective cohort 1997–2000) | LURIC Study— Winkelmann et al.[12] | LURIC biobank (https://www.luric.online) |
| Critical commercial assays | | |
| NT-proBNP electrochemiluminescence immunoassay | Roche Diagnostics | Assay family: Elecsys proBNP (ECLIA); legacy catalog numbers archived and available from the lead contact on request. |
| Factor XI activity, one-stage clotting assay | Diagnostica Stago | Assay family: human FXI-deficient substrate plasma; legacy catalog numbers archived and available from the lead contact on request. |
| Activated partial thromboplastin time (aPTT) reagent (kaolin/cephalin) | Diagnostica Stago | Assay family: STA APTT Kaolin; legacy catalog numbers archived and available from the lead contact on request. |
| Software and algorithms | | |
| R (4.3.1) | The R Project for Statistical Computing | https://www.r-project.org/ |
| survival (3.5–7) | Therneau T[13] | https://cran.r-project.org/package=survival |
| rms (6.7-1) | Harrell F[14] | https://cran.r-project.org/package=rms |
| ggplot2 (3.4.4) | Wickham H[15] | https://cran.r-project.org/package=ggplot2 |

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

We used data from the prospective Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Between 1997 and 2000, LURIC recruited patients undergoing invasive coronary diagnostics. The cohort and measurement procedures have been described previously by Winkelmann et al.[12] Inclusion required availability of a coronary angiogram and clinically stable status (except for acute coronary syndromes). As per original design, the cohort was restricted to native Germans. Exclusions included any acute non-cardiac illness, recent surgery (≤3 months), or a history of malignancy within 5 years. The study protocol was approved by the Ethics committee of the Landesärztekammer Rheinland-Pfalz (No. 1997-203); all volunteers provided written informed consent.

Sex assigned at birth (female/male), age, and ancestry (German) were collected via structured interviews and verified by study personnel. Gender identity was not collected. Information on occupation and socioeconomic status was obtained during standardized interviews. Information on deaths was obtained from local population registers, and causes of death were adjudicated using death certificates.

Of the initial 3,316 participants, 146 were excluded due to missing data in key variables. This included 32 individuals with FXI activity equal to 0%, which were assumed to reflect potential technical or pre-analytical errors. The distribution of FXI activity in the study cohort is shown in Figure S1. The final analytic sample comprised 3,170 participants with 69.2% males, had a median age of 63.6 [IQR 56.3–70.6] years and median BMI of 27.1 [IQR 24.7–29.7] kg/m$^2$ at recruitment. Out of 3170 patients, 2132 (67%) had been diagnosed with coronary artery disease (CAD), defined angiographically as at least one vessel with a minimum 50% stenosis. During a median follow-up of 14.5 years [IQR 8.5, 17.5], 1,640 persons deceased. Baseline characteristics with medians [IQR], proportions, and the number of missing values for each variable are provided in Table 1. The variables stratified by angiography-confirmed CAD, including medication use at baseline, are shown in Table S1.

We assessed effect modification by sex using an interaction between spline terms for FXI and sex in Cox models and found no evidence that the FXI–mortality association differed by sex (reported in the Results section). Gender identity was not collected, which may limit generalizability to gender-diverse populations.

## METHOD DETAILS

Fasting venous blood was drawn prior to angiography. Samples were processed within 15–20 min (serum after ~40 min), centrifuged 4°C/3800 rpm/15 min, aliquoted (1 mL), snap-frozen, and stored at −80°C.[12] Selected parameters were measured immediately in on-site labs.

FXI activity was measured in citrated plasma by a one-stage, aPTT-based clotting assay on an STA analyzer (Diagnostica Stago). Patient plasma was mixed with human FXI-deficient substrate plasma (supplier at the time: Immuno GmbH, Heidelberg, Germany) and clotting was initiated with an aPTT reagent (kaolin/cephalin lyophilisate); the reaction was recalcified with 0.025 mol/L $CaCl_2$, and clotting time was converted to % activity relative to a calibration curve prepared from pooled normal plasma, according to the manufacturer's instructions. This configuration (FXI-deficient plasma on STA with kaolin aPTT and 0.025 mol/L $CaCl_2$) is documented in the LURIC laboratory appendix.[12] Exact catalog numbers from the original measurements (1997–2000) are archived by the study laboratory and will be provided by the lead contact upon request; these are legacy catalog numbers, not listed in the original LURIC publications and may no longer be active.

NT-proBNP was quantified by electrochemiluminescence immunoassay (ECLIA) on a Roche Elecsys 2010 analyzer using the Elecsys proBNP assay, following the manufacturer's instructions; results were reported in ng/L.[16] Exact catalog numbers from the original measurements (1997–2000) are archived by the study laboratory and will be provided by the lead contact upon request; these are legacy catalog numbers, not listed in the original LURIC publications and may no longer be active.

## QUANTIFICATION AND STATISTICAL ANALYSIS

The primary objective was to characterize the potentially nonlinear association of FXI activity with all-cause mortality and to assess its variation by angiographic CAD, and NT-proBNP level. A sensitivity analysis evaluated major adverse cardiac events related mortality ($n$ = 3170, events = 590); methods were identical to the primary analysis and results are provided in Figure S3.

Follow-up time was calculated from the baseline visit to the date of death or last confirmation of survival. Time-to-event analyses used Cox proportional hazards models with time since baseline as the timescale. FXI activity was modeled with restricted cubic splines (RCS) to allow for non-linearity with knots at the 25th, 50th, and 75th percentiles of the FXI activity (89.50%, 111.67%, 139.06%).

Non-linearity was evaluated with Wald $\chi^2$ tests comparing the spline model to a linear-term-only model. Proportional-hazards assumptions were evaluated using Schoenfeld residual tests (global and term-specific) with inspection of log(−log) survival curves. Where proportional-hazards violations were detected for age and BMI, we fitted sensitivity models with time-varying coefficients (covariate×log(time)) to verify robustness of FXI estimates; FXI spline terms met proportional-hazards.

Analyses used complete cases for the variables included in each model. Core analyses required FXI activity, age, sex, BMI, survival time, and vital status (complete-case $n$ = 3,170, events = 1640). For extended covariate model, we additionally required non-HDL cholesterol, eGFR, and mean arterial pressure (complete-case $n$ = 3,165, events = 1637), and for NT-proBNP and CAD model NT-proBNP was required (complete-case $n$ = 3,123, events = 1611).

We fitted a global model including FXI activity (RCS) × CAD (yes/no) × NT-proBNP (continuous, ng/L), yielding all lower-order interactions. For interpretability in the figures, we also showed CAD-stratified FXI activity – hazard ratio curves. Within each CAD subset we displayed FXI activity – hazard ratio curves by NT-proBNP tertiles, defined with equal-frequency ranking (Table S2).

Analyses were performed in R 4.3.1[17] using *survival*[13] and *rms*[14]; figures were produced with *ggplot2*[15] (package versions listed in the key resources table).

Exact number of participants, event counts, non-linearity and interaction test results are reported in the figure legends, figures and Results.