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ORIGINAL RESEARCH

Screening of Multiple Biomarkers Associated With Ischemic Stroke in Atrial Fibrillation

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BACKGROUND: To explore the pathophysiological features of ischemic stroke in patients with atrial fibrillation (AF), we evaluated the association between 268 plasma proteins and subsequent ischemic stroke in 2 large AF cohorts receiving oral anticoagulation.

METHODS AND RESULTS: A case-cohort sample of patients with AF from the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial, including 282 cases with ischemic stroke or systemic embolism and a random sample of 4124 without these events, during 1.9 years of follow-up was used for identification. Validation was provided by a similar case-cohort sample of patients with AF from the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial, including 149 cases with ischemic stroke/systemic embolism and a random sample of 1062 without these events. In plasma obtained before randomization, 268 unique biomarkers were measured with OLINK proximity extension assay panels (CVD II, CVD III, and Inflammation) and conventional immunoassays. The association between biomarkers and outcomes was evaluated by random survival forest and adjusted Cox regression. According to random survival forest or Cox regression analyses, the biomarkers most strongly and consistently associated with ischemic stroke/systemic embolism were matrix metalloproteinase-9, NT-proBNP (N-terminal pro-B-type natriuretic peptide), osteopontin, sortilin, soluble suppression of tumorigenesis 2, and trefoil factor-3. The corresponding hazard ratios (95% CIs) for an interquartile difference were as follows: 1.18 (1.00–1.38), 1.55 (1.28–1.88), 1.28 (1.07–1.53), 1.19 (1.02–1.39), 1.23 (1.05–1.45), and 1.19 (0.97–1.45), respectively.

CONCLUSIONS: In patients with AF, of 268 unique biomarkers, the 6 biomarkers most strongly associated with subsequent ischemic stroke/systemic embolism represent fibrosis/remodeling (matrix metalloproteinase-9 and soluble suppression of tumorigenesis 2), cardiac dysfunction (NT-proBNP), vascular calcification (osteopontin), metabolism (sortilin), and mucosal integrity/ischemia (trefoil factor-3).

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Key Words: atrial fibrillation ■ biomarkers ■ ischemic stroke ■ pathophysiological features ■ screening

Atrial fibrillation (AF) is a common arrhythmia and constitutes a major health problem worldwide mainly because of its associated increased risk of stroke.[1] In individuals with AF, the risk of ischemic stroke is substantially reduced by oral anticoagulation; however, residual risk remains.[2,3] Several clinical characteristics are associated with an increased risk of ischemic stroke in patients with AF regardless of oral anticoagulation, most importantly older age, prior stroke, and cardiovascular comorbidity.[4,5] In recent years, protein biomarkers, such

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CLINICAL PERSPECTIVE

What Is New?

- In this study, the novel proximity extension assay protein screening technology was for the first time used for mass screening to identify biomarkers associated with ischemic stroke or systemic embolism during ongoing anticoagulation treatment in patients with atrial fibrillation.
- The identified biomarkers represent fibrosis/remodeling (matrix metalloproteinase-9 and soluble suppression of tumorigenesis 2), cardiac dysfunction (NT-proBNP [N-terminal pro-B-type natriuretic peptide]), vascular calcification (osteopontin), metabolism (sortilin), and mucosal integrity/ischemia (trefoil factor-3).

What Are the Clinical Implications?

- The results represent an important contribution in the step toward a better mechanistic understanding of ischemic stroke in patients with atrial fibrillation.
- These markers could help guide research into new therapeutic targets beyond anticoagulation in patients with atrial fibrillation at risk for stroke, and potentially even identify the population of patients who are at risk for thromboembolic stroke.

Nonstandard Abbreviations and Acronyms

ADAMTS13	a disintegrin and metalloproteinase with thrombospondin motifs 13
ARISTOTLE	Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation
cTnT-hs	high-sensitivity cardiac troponin T
MMP	matrix metalloproteinase
PEA	proximity extension assay
RE-LY	Randomized Evaluation of Long-Term Anticoagulation Therapy
SE	systemic embolism
sST2	soluble suppression of tumorigenesis 2
ST2	suppression of tumorigenesis 2
TFF3	trefoil factor 3

as cardiac troponin, reflecting myocardial damage, and NT-proBNP (N-terminal pro-B-type natriuretic peptide), reflecting cardiac stress and dysfunction, have been shown to be more important for prediction of ischemic stroke than all clinical information, except prior stroke, in patients with AF.[4,6] Biomarkers reflecting pathways of inflammation, renal function, coagulation, and platelet activity have also been investigated, although without consistent evidence of association with risk.[7] To better understand the remaining risk of ischemic stroke in anticoagulated patients with AF, there is a need to further explore additional mechanisms.

Recently, a highly sensitive proteomic assay platform has been developed, the proximity extension assay (PEA), which allows high-throughput multiplex screening of proteins in a resource-efficient procedure using small amounts of plasma.[8] We explored this new biomarker screening approach to identify plasma biomarkers associated with subsequent risk of ischemic stroke or systemic embolism (SE) and to improve the mechanistic understanding of ischemic stroke risk in patients with AF on oral anticoagulation.

METHODS

The data, analytical methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Patient Population

Identification Cohort

Details of the ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial have been published previously.[3,9] Briefly, the ARISTOTLE trial was a double-blind, double-dummy, randomized clinical trial that enrolled 18 201 patients with AF and at least 1 risk factor for stroke or SE between December 2006 and April 2010. Patients were randomized to warfarin (n=9081) or apixaban (n=9120). Exclusion criteria included conditions other than AF that required anticoagulation (eg, prosthetic heart valve) and severe renal insufficiency (serum creatinine >2.5 mg/dL [>221 μmol/L] or calculated creatinine clearance <25 mL/min).

Validation Cohort

The RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial was a prospective, multicenter, randomized trial comparing 2 blinded doses of dabigatran with open-label warfarin that enrolled 18 113 patients with AF between December 2005 and March 2009.[2,10] Exclusion criteria included severe heart valve disorder, recent stroke, creatinine clearance of <30 mL/min, or active liver disease.

In both trials, patients at certain centers participated in biomarker substudies and provided, before randomization, venous blood samples into vacutainer tubes containing EDTA, which were centrifuged immediately. Plasma was frozen in aliquots

and stored at –70°C until analyzed centrally at the Uppsala Clinical Research Center, an academic platform for analyses of biomarkers at the Uppsala University Hospital, Uppsala, Sweden. In both trials, ethics committee approval was obtained for all investigational sites, and all patients provided written informed consent.

Multimarker Screening Study Design

The inclusion of patients in this multimarker substudy was based on an unstratified case-cohort design. The ARISTOTLE trial multimarker subset consisted of all 282 cases with ischemic stroke or SE during follow-up of patients in the biomarker substudy, which is compared with a random sample of 4124 without these events. The RE-LY trial multimarker subset consisted of all 149 cases with ischemic stroke or SE and a random sample of 1062 without these events during follow-up of patients in the RE-LY trial biomarker substudy. As such, and in accordance with the traditional case-cohort method, individuals with events may be selected to the random sample of controls. The median follow-up time in both multimarker substudy cohorts was 1.9 years.

Outcome Assessment

All strokes were adjudicated by an international team of adjudicators blinded to treatment assignment.[2,3,9,10] Stroke was defined as the sudden onset of a focal neurologic deficit in a location consistent with the territory of a major cerebral artery and categorized as ischemic, hemorrhagic, or unspecified. SE was defined as an acute vascular occlusion of an extremity or organ, documented by means of imaging, surgery, or autopsy. The predefined primary outcome event for these substudies was ischemic stroke (including unspecified) or SE.

Biochemical Analyses

The plasma concentrations of high-sensitivity cardiac troponin T (cTnT-hs), NT-proBNP, and growth differentiation factor 15 (precommercial assay) were determined by Roche immunoassays using a Cobas Analytics e601 (Roche Diagnostics, Penzberg, Germany) and interleukin 6 high-sensitivity sandwich ELISA immunoassays (R&D Systems Inc, Minneapolis, MN). Cystatin C was analyzed with the ARCHITECT system ci8200 (Abbott Laboratories, Abbott Park, IL) using the particle-enhanced turbidimetric immunoassay from Gentian (Moss, Norway).[11] Estimated glomerular filtration rate was calculated on the basis of centrally determined creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration equation.[12]

The proteomic analyses were performed at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala University, without information on any other data. The determinations were performed using a high-throughput technique using the OLINK Proteomics Multiplex CVD II^{96x96}, CVD III^{96x96}, and Inflammation^{96x96} panels, which together simultaneously measured 276 selected proteins in plasma potentially related to cardiovascular disease and inflammation. The PEA technology uses a homogeneous assay that uses pairs of antibodies equipped with DNA reporter molecules. In the kits, 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target if present in the sample.[8,13] As only the correctly matched antibody pairs produce a signal, the technology has an exceptionally high specificity. When binding to their correct targets, they produce new DNA amplicons, with each identifier barcoding its respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark HD real-time polymerase chain reaction platform. The analyses were run using the internal controls for the PEA, including 2 incubation controls and extension and detection controls. For sample control in each plate, there is an interplate control used for normalization and it compensates for interplate variation. For each plate, a negative control, buffer without antigen, and 2 positive controls, pooled plasma, are used. All samples were analyzed in one set. Interplate variability was adjusted by intensity normalization. The resulting relative values, normalized protein expression data, were log2 transformed and a high value corresponded to a high protein concentration. For data analysis, the OLINK wizard was used and all statistical analyses were performed at Uppsala Clinical Research Center. The PEA assays have shown high reproducibility and repeatability, with mean intra-assay and interassay coefficients of variation around 8% and 12%, respectively; average intersite variation has been reported at 15%.[8] Prior validation studies have also showed that biomarkers analyzed with the PEA technique have an adequate concordance with conventional immunoassays.[14] The protein markers in the identification cohort included 3 panels, CVD II, CVD III, and Inflammation, and are detailed in Table S1 and S2. Of the 276 PEA proteins, 10 were available on >1 panel, resulting in 266 unique markers. As initial results in the identification cohort identified biomarkers from the CVD II and CVD III panels as more strongly associated with the outcome, the Inflammation panel was omitted in the external validation.

Statistical Analysis

The pairwise association between PEA biomarkers and established conventional biomarkers was assessed by the Spearman correlation.

A random survival forest algorithm[15] was used to evaluate the simultaneous association between ischemic stroke/SE and biomarkers. The evaluation included levels of 263 PEA markers, 4 conventional markers (NT-proBNP, cTnT-hs, growth differentiation factor 15, and interleukin 6), renal function, and 13 clinical characteristics (randomized treatment, age, sex, body mass index, smoking, hypertension, diabetes mellitus, hemoglobin, previous myocardial infarction, stroke/transient ischemic attack, peripheral artery disease, heart failure, and bleeding). The number of trees was 5000, splits were done according to a maximally selected statistic criterion, and the variables were ranked according to their permutation variable importance. Subjects with all PEA markers missing were excluded. There were only a few partially missing values, and these were singly imputed using multivariate imputations by chained equations.[16] Three PEA markers were omitted as they were also measured as conventional markers. An identical approach was used in the RE-LY trial evaluation, with a total of 184 PEA markers.

Cox regression analyses were performed, including each of the established standard immunoassays (naturally log transformed) and the PEA biomarkers, one at a time, assuming a linear association with the log hazard rate. Sampling weights were used to account for the case-cohort design. The randomly sampled controls were given weights equal to 1/0.2945632, corresponding to the reciprocal of the sampling probability of being selected for the PEA substudy. The Cox regression analyses were performed in 2 steps, first unadjusted and second adjusting for baseline characteristics (age, sex, body mass index, smoking, hypertension, diabetes mellitus, prior myocardial infarction, prior stroke/transient ischemic attack, peripheral artery disease, heart failure, and randomized treatment), renal function (cystatin C in ARISTOTLE trial and Chronic Kidney Disease Epidemiology Collaboration equation in RE-LY trial), and established biomarkers (NT-proBNP and cTnT-hs). Time to event was defined as the time since randomization until the occurrence of an ischemic stroke/SE or, if no ischemic stroke/SE occurs, the event is censored at last day of follow-up at the end of the study or death. Results were presented as the relative hazard for an interquartile difference of each marker with corresponding 95% CIs and *P* values. Thus, the hazard ratio (HR) can be interpreted as the relative hazard comparing the 2 biomarker values defining the inner 50% of the distribution (ie, the third versus the first quartile). On the inflammation panel, 16 of the proteins had >80% of the measurements below the limit of detection and these were not included in the Cox regression models.

Because of the large number of biomarkers evaluated, only biomarkers of high ranking in the random survival forest analysis (top 20) or with significant association in the adjusted Cox regression analysis, in both the identification and external validation cohorts, were considered to have confirmed association with the risk of ischemic stroke/SE.

All analyses were done using the R environment for statistical computing, version 3.3.1,[17] using the ranger[18] package.

RESULTS

Baseline Characteristics and Distribution of Biomarkers

The baseline characteristics of the multimarker substudy identification and validation cohorts are presented in Tables 1 and 2, respectively, according to occurrence of ischemic stroke/SE during follow-up. Baseline characteristics were similar between the identification and screening cohort, with only slight differences in regard to age and renal function (Tables 1 and 2). Patients with ischemic stroke/SE events were slightly older and to a larger extent had a history of stroke/transient ischemic attack. There were substantial differences in the levels of conventional biomarkers at baseline (eg, higher concentrations of NT-proBNP and cardiac troponin and lower estimated glomerular filtration rate in cases versus noncases). The relative concentration (normalized protein expression values) and limit of detection of all 266 biomarkers in the identification cohort are shown in Tables S1 and S2 for the external validation cohort. The biomarkers in general showed a low correlation with the established cardiovascular biomarkers. Correlation data for the biomarkers are presented in Tables S3 and S4, which show that several biomarkers were correlated with renal function.

Evaluation of Prognostic Biomarkers for Ischemic Stroke/SE

The median normalized protein expression values in both cohorts, divided by cases and noncases, are presented in Table S1 and S2.

In the identification cohort, of the 268 unique biomarkers and 13 clinical variables, the variables most strongly associated with ischemic stroke/SE in the random survival forest analysis are presented in Figure 1A. Among the biomarkers most strongly associated with the outcome, most were from the OLINK CVD II and CVD III panels. Thus, the Inflammation panel was not analyzed in the external validation cohort. The results from the validation process in

Table 1. Baseline Characteristics for the Identification Cohort

Variable	No Event (n=4124)	Ischemic Stroke/SE (n=282)
Age, y	70.0 (62.0–76.0)	71.0 (66.0–77.0)
Women	36.3 (1499)	41.8 (118)
Body mass index, kg/m^2	28.6 (25.4–32.7) [20]	27.7 (24.4–31.6) [1]
Current smoker	8.9 (367) [1]	8.2 (23) [0]
Hypertension	87.5 (3610)	88.3 (249)
Diabetes mellitus	25.1 (1035)	27.7 (78)
Prior myocardial infarction	13.1 (539)	16.3 (46)
Prior stroke/TIA	18.0 (743)	37.2 (105)
Peripheral arterial disease	4.7 (194)	8.2 (23)
Heart failure	31.1 (1282)	35.1 (99)
Hemoglobin, g/dL	14.2 (13.1–15.2) [22]	14.3 (13.2–15.2) [2]
NT-proBNP, ng/L	692.0 (363.0–1253.0) [3]	1014.0 (565.0–1773.2) [0]
cTnT-hs, ng/L	10.8 (7.4–16.6)	12.8 (9.0–20.6)
GDF-15, ng/L	1369.5 (965.0–2062.8)	1591.0 (1128.5–2318.2)
Cystatin C, mg/L	1.0 (0.8–1.2) [4]	1.1 (0.9–1.3) [0]
IL-6, ng/L	2.3 (1.5–4.0) [1]	2.7 (1.6–3.9) [0]
eGFR, CKD-EPI equation, mL/min	75.1 (57.1–96.8) [4]	67.6 (52.0–88.1) [0]

Continuous variables presented as median (quartile 1–quartile 3). Categorical variables presented as percentage (frequency). Number of missing values presented in brackets, and does not include cases where biomarker concentrations were below detection limit. CKD-EPI indicates Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; cTnT-hs, high-sensitivity cardiac troponin T; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, systemic embolism; and TIA, transient ischemic attack.

Table 2. Baseline Characteristics for the External Validation Cohort

Variable	No Event (n=1062)	Ischemic Stroke/SE (n=149)
Age, y	72.0 (67.0–77.0)	74.0 (69.0–80.0)
Women	37.1 (394)	37.6 (56)
Body mass index, kg/m^2	27.9 (25.0–31.3) [2]	27.1 (24.3–30.5) [0]
Current smoker	7.4 (79)	12.1 (18)
Hypertension	79.4 (843)	77.9 (116)
Diabetes mellitus	21.4 (227)	27.5 (41)
Prior myocardial infarction	16.9 (179)	18.1 (27)
Prior stroke/TIA	20.0 (212)	33.6 (50)
Peripheral arterial disease	3.6 (38)	4.7 (7)
Heart failure	28.9 (307)	34.9 (52)
Hemoglobin, g/dL	14.2 (13.1–15.3) [22]	14.1 (13.0–15.3) [3]
NT-proBNP, ng/L	839.5 (390.2–1457.5)	1156.0 (583.0–2127.0)
cTnT-hs, ng/L	12.1 (7.7–19.4)	14.2 (9.8–23.3)
GDF-15, ng/L	1470.0 (1095.2–2150.5)	1711.0 (1334.0–2618.0)
Cystatin C, mg/L	1.0 (0.8–1.2) [348]	1.1 (0.9–1.3) [41]
IL-6, ng/L	2.4 (1.5–3.9) [349]	2.8 (1.9–4.5) [41]
eGFR, CKD-EPI equation, mL/min	65.7 (54.9–76.9) [8]	61.8 (50.8–75.0) [1]

Continuous variables presented as median (quartile 1–quartile 3). Categorical variables presented as percentage (frequency). Number of missing values presented in brackets, and does not include cases where biomarker concentrations were below detection limit. CKD-EPI indicates Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; cTnT-hs, high-sensitivity cardiac troponin T; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; IL-6, interleukin 6; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SE, systemic embolism; and TIA, transient ischemic attack.

the external cohort, on the basis of 186 biomarkers and 12 clinical variables, are presented in Figure 2B. Among the top 20 markers in both cohorts, 5 variables (prior stroke, NT-proBNP, trefoil factor 3 [TFF3], soluble suppression of tumorigenesis 2 (sST2), and osteopontin) were consistently associated with ischemic stroke/SE.

The Cox analyses, evaluating each of the individual 255 biomarkers, identified 35 as statistically significantly associated with ischemic stroke/SE when adjusting for clinical characteristics, renal function (cystatin C), and cardiac biomarkers (NT-proBNP and cTnT-hs) in the identification cohort (Figure 2A, with unadjusted results in Figure S1A), and 20 in the validation cohort (Figure 2B, with unadjusted results in Figure S1B). Of these, 3 biomarkers were consistently significantly associated with ischemic stroke/SE in both cohorts (Table 3): NT-proBNP, sortilin, and matrix metalloproteinase (MMP)-9 (Table 3). For these biomarkers, the HR (95% CI) per interquartile range in the Cox regression analyses adjusted for clinical variables, renal function, and the cardiac biomarkers, was 1.55 (1.28–1.88) for NT-proBNP, 1.19 (1.02–1.39) for sortilin, and 1.18 (1.00–1.38) for MMP9. The corresponding HRs in the external validation cohort are presented in Table 3.

Among the few biomarkers associated with decreased risk of ischemic stroke/SE, according to both random survival forest and adjusted Cox regression analysis, was a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13); however, this finding was not consistent in the validation cohort (Table 3).

The correlation between these top candidate prognostic biomarkers and established cardiovascular (NT-proBNP and cTnT-hs) and renal biomarkers (cystatin C) is shown in Table 4. TFF3 was moderately correlated with renal function (ρ, 0.59). Beyond that, no strong patterns of correlation were seen (ρ, <0.5). The baseline concentrations of these top candidate prognostic biomarkers are summarized in Table S5, and their associations with ischemic stroke/SE, by using splines, are shown in Figure S2.

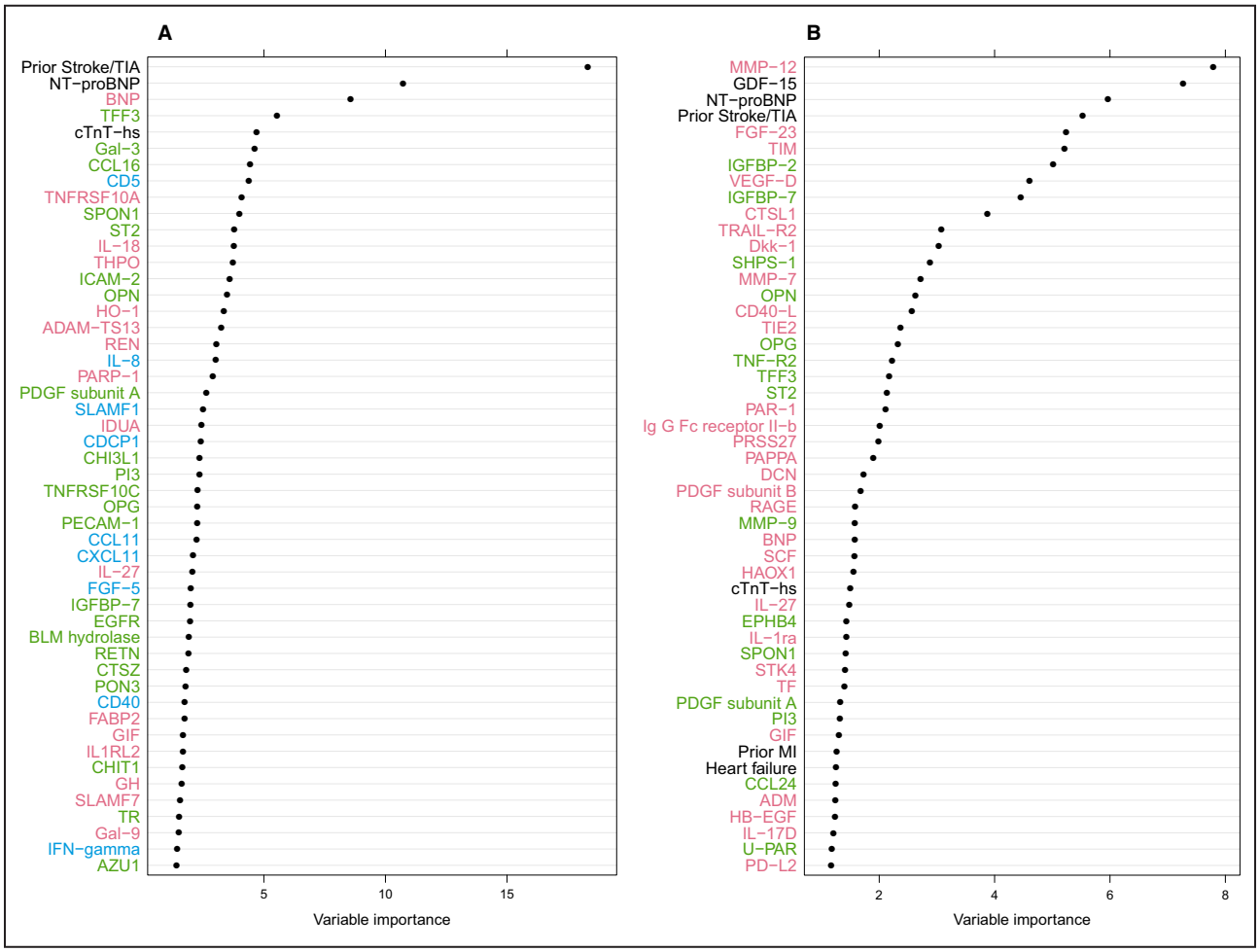


Figure 1. Variable importance for ischemic stroke/systemic embolism, according to random survival forest.
A, Identification cohort. Red indicates biomarkers analyzed on CVD II panel; green, biomarkers analyzed on CVD III panel; and blue, biomarkers analyzed on Inflammation panel. Biomarkers listed in black were analyzed with conventional immunoassays. Only the top 50 variables are shown. The evaluation included 263 proximity extension assay (PEA) markers, 4 conventional markers (NT-proBNP [N-terminal pro-B-type natriuretic peptide], high-sensitivity cardiac troponin T [cTnT-hs], growth differentiation factor 15 [GDF-15], and interleukin 6 [IL-6]), renal function, and 13 clinical characteristics. Protein names and UniProt numbers are found in Table S1. **B**, Validation cohort. Red indicates biomarkers analyzed on CVD II panel; green, biomarkders analyzed on CVD III panel; and blue, biomarkers analyzed on Inflammation panel. Biomarkers listed in black were analyzed with conventional immunoassays. Only the top 50 variables are shown. The evaluation included levels of 182 PEA markers, 4 conventional markers (NT-proBNP, cTnT-hs, GDF-15, and IL-6), renal function, and 13 clinical characteristics. Protein names and UniProt numbers are found in Table S2. MI indicates myocardial infarction; ST2, suppression of tumorigenesis 2; and TIA, transient ischemic attack.

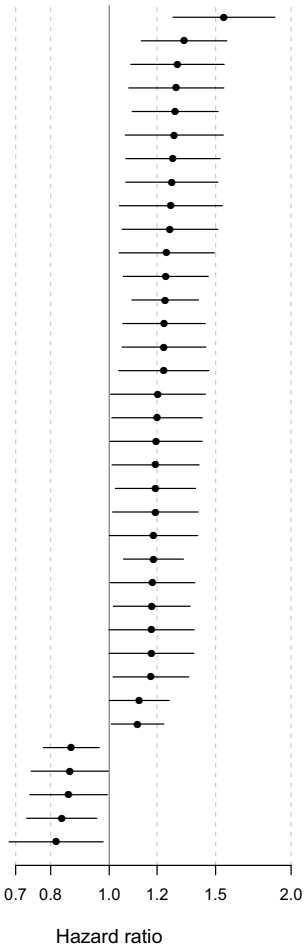
DISCUSSION

In the present study, on the basis of 2 separate cohorts with anticoagulated patients with AF, a comprehensive and systematic screening of 268 protein biomarkers to identify those associated with ischemic stroke/SE was performed. An unbiased evaluation of the most important prognostic variables

Figure 2. Forest plot of biomarkers associated with ischemic stroke or systemic embolism, according to adjusted Cox regression analysis.
A, Identification cohort. A forest plot showing all 255 biomarkers is available in Figure S1. Red indicates biomarkers analyzed on CVD II panel; green, biomarkers analyzed on CVD III panel; and blue, biomarkers analyzed on Inflammation panel. Biomarkers listed in black were analyzed with conventional immunoassays. Model adjusted for baseline characteristics, renal function, and cardiac biomarkers (NT-proBNP [N-terminal pro-B-type natriuretic peptide] and high-sensitivity cardiac troponin T [cTnT-hs]). Low and High correspond to the first and third sample quartiles of the respective biomarkers. Protein names and UniProt numbers are found in Table S1. **B**, Validation cohort. Red indicates biomarkers analyzed on CVD II panel; and green, biomarkers analyzed on CVD III panel. Biomarkers listed in black were analyzed with conventional immunoassays. Model adjusted for baseline characteristics, renal function, and cardiac biomarkers (NT-proBNP and cTnT-hs). Low and High correspond to the first and third sample quartiles of the respective biomarkers. Protein names and UniProt numbers are found in Table S2. HR indicates hazard ratio; and ST2, suppression of tumorigenesis 2.

A

	Low	High	HR [95% CI]	P
NT−proBNP (ng/L)	404	1438	1.549 [1.275, 1.880]	1.004e−05
CCL16	5.784	6.494	1.330 [1.130, 1.566]	6.046e−04
TFPI	8.356	8.828	1.297 [1.086, 1.550]	4.158e−03
CTSZ	4.775	5.303	1.291 [1.077, 1.548]	5.748e−03
vWF	5.854	7.059	1.285 [1.091, 1.514]	2.638e−03
cTnT−hs (ng/L)	7.9	18.38	1.282 [1.063, 1.545]	9.237e−03
OPN	5.034	5.796	1.275 [1.065, 1.525]	8.002e−03
LDL receptor	4.17	5.02	1.269 [1.065, 1.513]	7.818e−03
RARRES2	11.88	12.23	1.265 [1.039, 1.539]	1.901e−02
PAI	5.591	6.889	1.261 [1.050, 1.514]	1.291e−02
TGM2	7.805	8.776	1.245 [1.038, 1.493]	1.814e−02
BLM hydrolase	5.505	6.006	1.241 [1.055, 1.459]	9.018e−03
IL−8	5.97	6.77	1.238 [1.090, 1.406]	9.890e−04
t−PA	5.892	6.667	1.232 [1.053, 1.442]	9.091e−03
ST2	3.894	4.61	1.232 [1.050, 1.446]	1.054e−02
CTSL1	5.767	6.452	1.231 [1.037, 1.462]	1.769e−02
ICAM−2	5.003	5.533	1.204 [1.005, 1.443]	4.384e−02
THPO	1.932	2.35	1.200 [1.010, 1.426]	3.810e−02
RETN	6.534	7.257	1.196 [1.003, 1.425]	4.569e−02
EGFR	1.759	2.047	1.193 [1.011, 1.409]	3.692e−02
SORT1	6.207	6.543	1.193 [1.024, 1.390]	2.352e−02
PECAM−1	4.542	5.01	1.192 [1.013, 1.404]	3.452e−02
CD163	7.613	8.245	1.184 [1.001, 1.401]	4.883e−02
MCP−3	1.92	2.46	1.184 [1.056, 1.327]	3.818e−03
PI3	3.786	4.68	1.180 [1.004, 1.386]	4.428e−02
CASP−8	2.36	3.53	1.176 [1.016, 1.361]	2.992e−02
AXL	7.925	8.397	1.175 [0.999, 1.383]	5.159e−02
MMP−9	3.71	4.736	1.175 [1.000, 1.381]	5.041e−02
SPON1	2.107	2.553	1.172 [1.014, 1.355]	3.119e−02
PARP−1	3.021	3.819	1.121 [1.000, 1.257]	4.975e−02
SLAMF7	2.387	2.793	1.114 [1.008, 1.232]	3.441e−02
ADAM−TS13	5.078	5.268	0.865 [0.778, 0.963]	7.921e−03
GIF	4.936	6.204	0.860 [0.743, 0.997]	4.492e−02
IL−17D	2.573	2.956	0.857 [0.739, 0.993]	4.034e−02
Protein BOC	4.686	5.043	0.834 [0.730, 0.954]	8.004e−03
REN	6.796	8.21	0.817 [0.683, 0.977]	2.689e−02



B

	Low	High	HR [95% CI]	P
NT−proBNP (ng/L)	435	1696	1.485 [1.118, 1.972]	0.00633
TIM	7.047	8.09	1.470 [1.174, 1.841]	0.00079
MMP−12	6.607	7.646	1.406 [1.105, 1.789]	0.00551
Ig G Fc receptor II−b	2.569	3.899	1.396 [1.048, 1.859]	0.02266
Gal−9	7.574	8.029	1.346 [1.038, 1.745]	0.02475
SHPS−1	3.339	3.943	1.336 [1.047, 1.704]	0.01995
MMP−9	4.583	5.698	1.320 [1.057, 1.648]	0.01443
IL−1RT1	6.185	6.607	1.301 [1.045, 1.621]	0.01857
PDGF subunit B	7.825	9.586	1.293 [1.022, 1.636]	0.03226
ALCAM	7.009	7.36	1.287 [1.032, 1.606]	0.02540
PD−L2	2.936	3.414	1.287 [1.070, 1.547]	0.00738
PRSS27	8	8.658	1.281 [1.009, 1.627]	0.04231
IL−1ra	4.217	5.192	1.268 [1.022, 1.573]	0.03113
GDF−15	5.99	6.813	1.266 [1.005, 1.595]	0.04531
PDGF subunit A	2.531	3.807	1.261 [1.018, 1.562]	0.03406
CD40−L	3.046	4.592	1.255 [1.046, 1.506]	0.01458
Dkk−1	8.074	8.916	1.248 [1.020, 1.528]	0.03142
SORT1	8.356	8.739	1.245 [1.028, 1.507]	0.02501
IL−18	8.236	8.986	1.194 [1.005, 1.418]	0.04410
GT	1.506	2.421	0.665 [0.516, 0.856]	0.00152

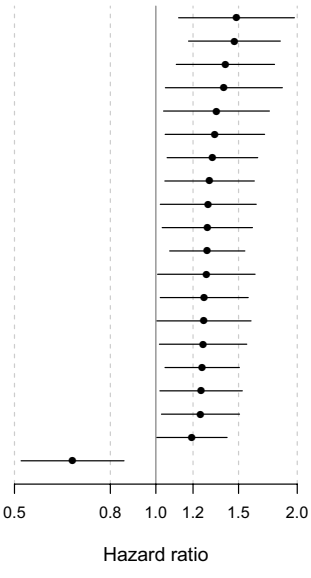


Table 3. Biomarkers Consistently Associated With Ischemic Stroke/SE, According to RF or Adjusted Cox Regression Analyses

Variable	RF Ranking	Low	High	Cox Model A		Cox Model B	
				Hazard Ratio (95% CI)	*P* Value	Hazard Ratio (95% CI)	*P* Value
Identification cohort							
NT-proBNP	1	404	1438	1.646 (1.379–1.963)	3.19E-08*	1.549 (1.275–1.880)	1.00361E-05
TFF3	3	5.338	6.043	1.278 (1.098–1.488)	0.00153	1.188 (0.974–1.450)	0.0899
ST2	10	3.894	4.61	1.419 (1.231–1.635)	1.44E-06	1.232 (1.050–1.446)	0.0105
Osteopontin	14	5.034	5.796	1.574 (1.347–1.838)	1.09E-08	1.275 (1.065–1.525)	0.0080
ADAMTS13	16	5.078	5.268	0.856 (0.779–0.941)	0.00130	0.865 (0.778–0.963)	0.00792
Sortilin	116	6.207	6.543	1.257 (1.089–1.451)	0.00182	1.193 (1.024–1.390)	0.0235
MMP9	183	3.71	4.736	1.213 (1.036–1.420)	0.0165	1.175 (1.000–1.381)	0.0504
External validation cohort							
NT-proBNP	3	435	1696	1.736 (1.342–2.246)	2.65E-05	1.485 (1.118–1.972)	0.00633
Osteopontin	14	7.127	7.936	1.426 (1.130–1.798)	0.00276	1.156 (0.878–1.521)	0.302
TFF3	19	4.856	5.551	1.354 (1.142–1.606)	0.0005	1.041 (0.783–1.384)	0.780
ST2	20	4.16	4.887	1.179 (0.954–1.457)	0.128	1.020 (0.803–1.296)	0.869
MMP9	28	4.583	5.698	1.297 (1.051–1.601)	0.01547	1.320 (1.057–1.648)	0.01443
Sortilin	76	8.356	8.739	1.310 (1.090–1.574)	0.00405	1.245 (1.028–1.507)	0.02501

Biomarkers identified as top markers by 2 different statistical methods, an RF (top 20 biomarkers) or an adjusted Cox regression analysis (model B; $P \leq 0.05$), were included in the table.

Analyses based on 4075 patients and 261 events, with all covariates available in the identification cohort, and 1196 patients and 129 events in the external validation.

Model A: Cox regression model adjusted for baseline characteristics: age, sex, body mass index, smoking, hypertension, diabetes mellitus, prior myocardial infarction, prior stroke/transient ischemic attack, peripheral artery disease, heart failure, and randomized treatment. Model B: same as A with addition of renal and cardiac biomarkers (NT-proBNP and high-sensitivity cardiac troponin T).

Hazard ratios per interquartile range. Figure S2 shows unadjusted nonlinear associations of these biomarkers with ischemic stroke/SE.

ADAMTS13 indicates a disintegrin and metalloproteinase with thrombospondin motifs 13; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RF, random survival forest; SE, systemic embolism; ST2, suppression of tumorigenesis 2; and TFF3, trefoil factor-3.

by a random survival forest approach and traditional Cox regression analyses identified NT-proBNP and 5 novel biomarkers (MMP9, osteopontin, sortilin, sST2, and TFF3) as having independent association with ischemic stroke/SE in patients with AF on oral anticoagulation. Furthermore, one additional biomarker may be of potential interest, as it was associated with reduced risk: ADAMTS13. These findings provide new opportunities to improve the mechanistic understanding of ischemic stroke in patients with AF as well as further refining risk assessment and clinical decision making in these patients.

Candidate Biomarkers and Potential Mechanisms

NT-proBNP demonstrated the strongest and most consistent association with ischemic stroke/SE. NT-proBNP is a previously established risk marker in cardiovascular disease and widely integrated in healthcare systems worldwide.[6] This study provides strong evidence that the association is not only robust, but it is independent of a comprehensive set of other protein cardiovascular biomarkers. Natriuretic peptides have repeatedly shown independent association with stroke, other cardiovascular outcomes, and death in many patient settings, including AF.[6] NT-proBNP has been suggested to be of atrial origin in AF because of myocyte stress in the atria, reflecting atrial dysfunction, which is an established risk factor for thrombosis formation in AF. This may be one plausible mechanism for the relation between NT-proBNP and thrombosis.[17] However, the exact mechanism behind the association with ischemic stroke in AF is still elusive, although several possible concepts have been suggested previously and revolve around pathways of myocardial damage.[19]

Recently, the biomarker was also included in a biomarker-based risk score for stroke in AF and has also been proposed for possible use for further refinement of stroke risk in international AF guidelines.[4,5]

Among the 5 newly identified biomarkers showing consistent association with ischemic stroke, TFF3 and osteopontin were moderately correlated and sortilin, sST2, and MMP9 were weakly correlated with established cardiovascular biomarkers, such as troponin and natriuretic peptides. Thus, they appear to represent different pathways, including metabolism, mucosal integrity, remodeling/fibrosis, and vascular calcification (Figure 3).

Table 4. Spearman Correlation Between the Top Novel Prognostic Candidate PEA Biomarkers and Established Biomarkers

Biomarker	Cystatin C	NT-proBNP	Troponin T
MMP9	0.14	0.03	0.06
Osteopontin	0.37	0.283	0.34
Sortilin	0.10	0.17	0.13
ST2	0.13	0.23	0.23
TFF3	0.59	0.34	0.44
ADAMTS13	-0.13	-0.11	-0.14

On the basis of the random identification cohort only (N=4075), excluding enriched cases.

PEA biomarkers in normalized protein expression values. Established biomarkers (cystatin C, NT-proBNP, and high-sensitivity troponin T) in logarithmic transformation of the ng/L level. Additional data on correlation for PEA biomarkers, identified in adjusted Cox analyses, are presented in Table S3 and S4.

Table S5 shows the baseline concentrations of the identified PEA biomarkers. ADAMTS13 indicates a disintegrin and metalloproteinase with thrombospondin motifs 13; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PEA, proximity extension assay; ST2, suppression of tumorigenesis 2; and TFF3, trefoil factor-3.

MMP9 belongs to a family of zinc-dependent endopeptidases involved in tissue remodeling.[20] MMP9, previously known as collagenase or gelatinase B, directly degrades extracellular matrix proteins and has been widely studied. It is secreted by several cells, most prominently neutrophils, fibroblasts, and macrophages.[20] In cardiovascular disease, MMP9 has been associated with hypertension, arterial stiffness, and cardiac hypertrophy.[20,21] MMPs have often been used as markers for myocardial fibrosis and have been associated with poorer prognosis in several cardiovascular settings.[22–24] However, the role of MMPs in AF has been less studied. Concentrations of MMP9 and other MMPs have been described to be higher in patients with persistent AF compared with controls in sinus rhythm.[25,26] The present results, to our knowledge, describe for the first time the prognostic role of MMP9 as a risk marker for ischemic stroke in patients with AF. The relation may be through potential direct mechanisms because these extracellular matrix degradation proteinases have previously been associated with left atrial dilatation, or potentially by less specific pathways reflecting a general burden of cardiac fibrosis and cardiomyopathy.[22]

Osteopontin is a noncollagenous bone matrix protein synthesized by osteoblasts and osteocytes.[27,28] It is, however, also expressed and secreted by a large number of other cells, such as neutrophils, fibroblasts, and myoblasts.[27,28] Osteopontin has been described to be a multifunctional cytokine involved in many physiological and pathological processes, including inflammation.[27,28] In cardiology, osteopontin has been described to be a mediator of cardiac fibrosis, with higher concentrations in the presence of cardiac fibrosis.[27] Osteopontin has also been suggested to be involved in vascular calcification and coronary atherosclerosis and associated with cardiovascular events, such as myocardial infarction and cardiovascular death.[29] Not much is known about osteopontin in the setting of AF. However, recently, osteopontin was described to be associated with atrial fibrosis[30] and identified as a prognostic biomarker for incident AF in community-dwelling adults in a protein profiling program.[31] The association of osteopontin with ischemic stroke/SE in patients with AF is novel.

Sortilin is expressed in several tissues and belongs to a family of Vps10p-domain receptors.[32] Sortilin has been described to be involved in the hepatic metabolism of low-density lipoproteins.[33] In several genome-wide association studies, the sortilin locus has consistently been associated with low-density lipoprotein concentrations.[34,35] Sortilin has therefore been suggested as a lipoprotein receptor that mediates the uptake of low-density lipoprotein particles into cells.[35] In addition, sortilin has been indicated to play a role in vascular calcification and potentially also in neuronal apoptosis.[32,33] The role of sortilin in AF has, to our knowledge, not been investigated previously. The present results show sortilin to be a consistent risk marker of ischemic stroke in AF. It is unclear if it plays a casual role through its involvement in calcification or exhibits secondary relations by pathways of atherosclerotic disease via lipid metabolism. Potentially, multiple effects may also be involved as a soluble form of sortilin is released from activated platelets.[36]

Among the newly identified biomarkers associated with ischemic stroke/SE, sST2 is probably the most recognized and most explored in the cardiovascular field.[37] Similar to the natriuretic peptides, the main focus of research on sST2 has been in heart failure. Suppression of tumorigenesis 2 (ST2) is a member of the interleukin 1 receptor family, with 2 main isoforms: transmembrane or cellular and soluble or circulating (sST2) forms. sST2 is considered to be a marker of myocardial stress, fibrosis, and remodeling.[38] Interleukin 33 binds to the transmembrane form of ST2 and has antihypertrophic and antifibrotic effects. The soluble form of ST2 (sST2), however, acts as a decoy receptor and blocks the cardioprotective effects. It is plausible that sST2 is associated with stroke in patients with AF in part via a similar pathophysiologic pathway as for the natriuretic peptides. However, beyond its myocardial role, the interleukin 33/ST2 system has recently also been shown to induce tissue factor, the main initiator of blood coagulation, expression and activity, in a subset of human monocytes and monocyte-derived procoagulant microvesicles and thus possibly represents another pathway for ischemic stroke/SE in patients with AF.[39] Recently, sST2 was shown to be associated with incident stroke in ambulatory individuals from the

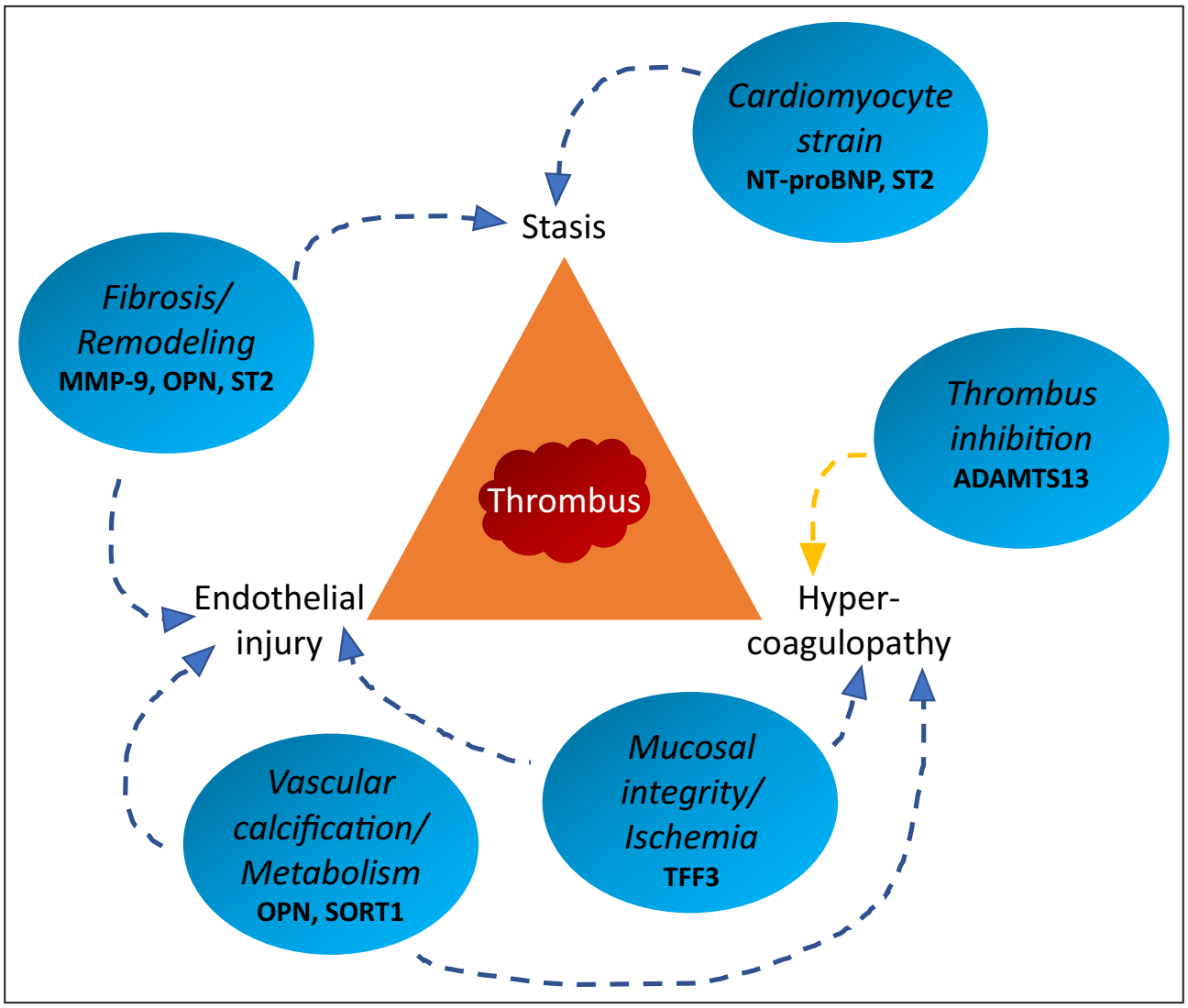


Figure 3. Illustration of putative pathways for the novel candidate biomarkers in relation to a model based on the Virchow triad for thrombus formation.
ADAMTS13 indicates a disintegrin and metalloproteinase with thrombospondin motifs 13; MMP-9, matrix metalloproteinase-9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPN, osteopontin; SORT1, sortilin; ST2, suppression of tumorigenesis 2; and TFF3, trefoil factor 3.

Framingham Offspring cohort.[40] The present results thus for the first time extend these observations to a population with AF.

TFF3, a member of the trefoil family, is mainly secreted from goblet cells in the gastrointestinal system and is involved in supporting mucosal integrity.[41] TFF3 has been associated with inflammation and different types of malignancies, as both conditions carry higher TFF3 concentrations. Studies of TFF3 in cardiovascular disease are limited.[41,42] Some studies have demonstrated increased concentrations in states of myocardial ischemia, and TFF3 has also been associated with cardioprotective effects in experimental animal models.[43] Its potential role in AF is thus novel. It may, however, be possible that TFF3 is simply a marker of underlying myocyte damage/ischemia rather than possessing direct causal effects in thrombus formation and ischemic stroke/SE in AF.

Of the 255 biomarkers in the identification cohort, few were associated with a reduced risk of ischemic stroke/SE. Among the biomarkers most strongly associated with lower risk of ischemic stroke/SE in this cohort with AF was ADAMTS13, also known as von Willebrand factor–cleaving protease. ADAMTS13 cleaves von Willebrand factor multimers into smaller less procoagulant forms and thereby exerts an inhibitory effect on platelet thrombus formation.[44] Low concentration of ADAMTS13 has also been associated with increased atherosclerosis in animal models.

Some smaller case-control studies in humans have shown low concentrations of ADAMTS13 to be associated with higher risk of ischemic stroke; however, inconsistencies exist.[44] However, the result concerning ADAMTS13 needs to be interpreted with caution because it could not be confirmed in the external validation cohort. Still, the rather unique association of this biomarker with lower risk of stroke may merit it for more in-depth studies with quantitative assays in prospective materials in the efforts to further understand and mitigate the risk of stroke in AF.

Implications

In this study, the novel PEA protein screening technology was, for the first time, used for mass screening to identify biomarkers associated with ischemic stroke or SE during ongoing anticoagulation treatment in patients with AF. Several biomarkers, reflecting different pathophysiological pathways for ischemic stroke/SE in AF, were consistently identified. The present results thus represent an important contribution in the step toward a better mechanistic understanding of ischemic stroke in patients with AF. These markers could help guide research into new therapeutic targets beyond anticoagulation in patients with AF at risk for stroke, and potentially even identify a population of patients (including those without clinical AF) who are at risk for thromboembolic stroke. The novel candidate biomarkers therefore need to be further evaluated using quantitative assays in prospective materials, and evaluated with mendelian randomization analyses and functional studies to better understand their pathophysiological links and causality to the disease processes.

Strengths and Limitations

The use of a well-defined clinical cohort with complete follow-up data and a stringent method of statistical evaluation applied, using 2 separate cohorts for an identification and validation process, strengthens the results. This approach therefore provides a large degree of certitude about the identified novel prognostic biomarkers. Also, 2 different statistical methods were used. The random survival forest treated all variables simultaneously and allowed, inherently, for nonlinear associations and complex interactions among the variables. The Cox regression, on the other hand, assumed a linear association between the log relative hazard of ischemic stroke/SE and each marker one at a time, making it possible to estimate average adjusted HRs in a more conventional way. The 2 methods thus captured different aspects of the possibly complex relationship between the biomarkers and the risk for ischemic stroke/SE and by that complemented each other in the process of screening for top biomarkers. Because of the high number of biomarkers in relation to number of events, the analyses focused on taking advantage of the availability of 2 separate AF cohorts instead of using specific methods to account for multiple testing, such as Bonferroni. There were slight differences between the 2 cohorts in regard to age and renal function. This may influence the results to some degree. Likewise, 3 panels were initially used in the identification cohort, and only 2 panels, containing more prognostically relevant biomarkers, were used in the external validation cohort. Thus, it is possible that some additional biomarkers of potential interest remained unconfirmed despite the thorough evaluation. For instance, this includes ADAMTS13 or spondin-1 in the identification cohort and T-cell immunoglobulin mucin receptor 1 or MMP12 in the validation cohort, as these biomarkers only showed strong associations with ischemic stroke in 1 of the 2 cohorts. Also, all patients were on oral anticoagulation and the results may thus not be fully generalizable to patients without antithrombotic treatment, although this data set may be especially valuable to explore “residual risk.” For the PEA method, a limitation is the lack of absolute values; however, prior validation studies have shown that biomarkers analyzed with the PEA technique have an adequate concordance with conventional immunoassays.[14]

CONCLUSIONS

In patients with AF on oral anticoagulation, of 268 biomarkers, 1 established biomarker, NT-proBNP, and 5 novel biomarkers, representing different pathophysiological pathways, showed consistent association with the risk of ischemic stroke/SE; MMP9 and sST2 (remodeling/fibrosis), osteopontin (vascular calcification), sortilin (metabolism), and TFF3 (mucosal integrity). Further evaluation of these novel biomarkers and pathways associated with ischemic stroke in patients with AF is warranted.

ARTICLE INFORMATION

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Supplementary Material

Tables S1–S5
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Baseline NPX values (arbitrary units) and limit of detection (LoD) of proximity extension assay (PEA) biomarkers in the identification cohort.

Variable		UniProt No.	No	Ischemic stroke/SE	LoD
ACE2	Angiotensin-converting enzyme 2	Q9BYF1	3.9 (3.5 -- 4.4) [203]	4.0 (3.6 -- 4.5) [12]	1.1
ADAM-TS13	A disintegrin and metalloproteinase with thrombospondin motifs 13	Q76LX8	5.2 (5.1 -- 5.3) [203]	5.2 (5.1 -- 5.2) [12]	1.4
ADM	ADM	P35318	7.5 (7.2 -- 7.8) [203]	7.6 (7.3 -- 7.9) [12]	1.9
AGRP	Agouti-related protein	O00253	3.2 (3.0 -- 3.5) [203]	3.3 (3.0 -- 3.6) [12]	0.7
AMBP	Protein AMBP	P02760	7.1 (7.0 -- 7.2) [203]	7.1 (7.0 -- 7.3) [12]	1.3
ANG-1	Angiopoietin-1	Q15389	8.9 (8.1 -- 9.6) [203]	9.0 (8.1 -- 9.8) [12]	2.2
BMP-6	Bone morphogenetic protein 6	P22004	5.7 (5.4 -- 6.0) [203]	5.8 (5.5 -- 6.1) [12]	1.4
BNP	Natriuretic peptides B	P16860	4.0 (3.0 -- 4.9) [203]	4.5 (3.7 -- 5.4) [12]	1.6
CA5A	Carbonic anhydrase 5A, mitochondrial	P35218	2.6 (2.1 -- 3.3) [203]	2.7 (2.2 -- 3.3) [12]	1.3
CCL17	C-C motif chemokine 17	Q92583	6.9 (6.3 -- 7.6) [203]	6.9 (6.3 -- 7.7) [12]	1.7
CCL3	C-C motif chemokine 3	P10147	2.8 (2.5 -- 3.1) [203]	2.9 (2.6 -- 3.2) [12]	1.6
CD4	T-cell surface glycoprotein CD4	P01730	4.7 (4.5 -- 4.9) [203]	4.7 (4.5 -- 5.0) [12]	1.3
CD40-L	CD40 ligand	P29965	4.1 (3.5 -- 5.1) [203]	4.2 (3.6 -- 5.1) [12]	1.2
CD84	SLAM family member 5	Q9UIB8	5.3 (5.1 -- 5.6) [203]	5.4 (5.1 -- 5.7) [12]	1.4
CEACAM8	Carcinoembryonic antigenrelated cell adhesion molecule 8	P31997	4.2 (3.9 -- 4.7) [203]	4.3 (3.9 -- 4.7) [12]	1.6
CTRC	Chymotrypsin C	Q99895	10.2 (9.7 -- 10.7) [203]	10.1 (9.7 -- 10.6) [12]	2.6
CTSL1	Cathepsin L1	P07711	6.1 (5.7 -- 6.4) [203]	6.1 (5.9 -- 6.6) [12]	0.9
CXCL1	C-X-C motif chemokine 1	P09341	8.3 (7.4 -- 8.9) [203]	8.2 (7.5 -- 8.9) [12]	2.7
DCN	Decorin	P07585	5.4 (5.3 -- 5.6) [203]	5.5 (5.3 -- 5.6) [12]	3.6
DECR1	2,4-dienoyl-CoA reductase, mitochondrial	Q16698	3.1 (2.5 -- 3.8) [203]	3.1 (2.5 -- 4.0) [12]	2.0
Dkk-1	Dickkopf-related protein 1	O94907	8.9 (8.5 -- 9.3) [203]	9.0 (8.6 -- 9.4) [12]	2.1
FABP2	Fatty acid-binding protein, intestinal	P12104	9.2 (8.6 -- 9.7) [203]	9.2 (8.6 -- 9.7) [12]	2.1
FGF-21	Fibroblast growth factor 21	Q9NSA1	7.7 (6.8 -- 8.6) [203]	7.9 (7.0 -- 8.8) [12]	2.6

FGF-23	Fibroblast growth factor 23	Q9GZV9	4.3 (3.8 -- 4.8) [203]	4.3 (4.0 -- 4.9) [12]	1.4
FS	Follistatin	P19883	12.0 (11.7 -- 12.3) [203]	12.0 (11.8 -- 12.3) [12]	3.6
Gal-9	Galectin-9	O00182	7.0 (6.8 -- 7.2) [203]	7.0 (6.8 -- 7.2) [12]	1.5
GDF-2	Growth/differentiation factor 2	Q9UK05	4.6 (4.2 -- 4.9) [203]	4.6 (4.2 -- 5.0) [12]	1.5
GH	Growth hormone	P01241	8.5 (7.0 -- 10.1) [203]	8.9 (7.6 -- 10.1) [12]	1.8
GIF	Gastric intrinsic factor	P27352	5.6 (5.0 -- 6.2) [203]	5.5 (4.8 -- 6.1) [12]	1.4
GLO1	Lactoylglutathione lyase	Q04760	6.1 (5.7 -- 6.7) [203]	6.2 (5.7 -- 6.7) [12]	1.9
GT	Gastrotropin	P51161	1.9 (1.6 -- 2.4) [203]	1.9 (1.6 -- 2.4) [12]	1.2
HAOX1	Hydroxyacid oxidase 1	Q9UJM8	4.6 (3.7 -- 5.7) [203]	4.7 (3.7 -- 5.7) [12]	1.1
HB-EGF	Proheparin-binding EGF-like growth factor	Q99075	5.8 (5.6 -- 6.1) [203]	5.9 (5.6 -- 6.1) [12]	0.8
HO-1	Heme oxygenase 1	P09601	11.7 (11.4 -- 11.9) [203]	11.6 (11.4 -- 12.0) [12]	4.4
hOSCAR	Osteoclast-associated immunoglobulin-like receptor	Q8IYS5	9.8 (9.6 -- 9.9) [203]	9.8 (9.6 -- 9.9) [12]	3.0
HSP 27	Heat shock 27 kDa protein	P04792	10.2 (9.6 -- 10.6) [203]	10.2 (9.5 -- 10.6) [12]	2.4
IDUA	Alpha-L-iduronidase	P35475	4.6 (4.1 -- 5.0) [203]	4.5 (4.1 -- 4.9) [12]	1.6
Ig G Fc receptor II-b	Low affinity immunoglobulin gamma Fc region receptor II-b	P31994	1.7 (1.4 -- 2.1) [203]	1.8 (1.4 -- 2.1) [12]	1.3
IL-17D	Interleukin-17D	Q8TAD2	2.7 (2.6 -- 2.9) [203]	2.8 (2.6 -- 2.9) [12]	1.2
IL-18	Interleukin-18	Q14116	8.7 (8.4 -- 9.1) [203]	8.8 (8.3 -- 9.2) [12]	2.5
IL-1ra	Interleukin-1 receptor antagonist protein	P18510	4.5 (4.1 -- 5.0) [203]	4.7 (4.2 -- 5.1) [12]	2.4
IL-4RA	Interleukin-4 receptor subunit alpha	P24394	3.2 (2.9 -- 3.5) [203]	3.3 (3.0 -- 3.6) [12]	1.2
IL-6	Interleukin-6	P05231	4.0 (3.5 -- 4.6) [203]	4.1 (3.6 -- 4.5) [12]	1.3
IL16	Pro-interleukin-16	Q14005	5.3 (5.0 -- 5.6) [203]	5.3 (5.0 -- 5.7) [12]	1.0
IL1RL2	Interleukin-1 receptor-like 2	Q9HB29	4.6 (4.3 -- 4.9) [203]	4.7 (4.3 -- 4.9) [12]	1.5
IL-27	Interleukin-27	Q8NEV9, Q14213	4.5 (4.2 -- 4.7) [203]	4.5 (4.3 -- 4.8) [12]	1.9
ITGB1BP2	Melusin	Q9UKP3	3.0 (3.0 -- 4.5) [203]	3.1 (3.0 -- 4.8) [12]	3.0
LEP	Leptin	P41159	6.7 (6.0 -- 7.4) [203]	6.7 (5.9 -- 7.3) [12]	2.2
LOX-1	Lectin-like oxidized LDL receptor 1	P78380	6.9 (6.5 -- 7.3) [203]	6.9 (6.6 -- 7.4) [12]	2.7
LPL	Lipoprotein lipase	P06858	9.5 (9.2 -- 9.8) [203]	9.6 (9.3 -- 9.8) [12]	2.7

MARCO	Macrophage receptor MARCO	Q9UEW3	6.2 (6.1 -- 6.4) [203]	6.2 (6.1 -- 6.4) [12]	1.3
MERTK	Tyrosine-protein kinase Mer	Q12866	4.5 (4.2 -- 4.7) [203]	4.5 (4.3 -- 4.7) [12]	1.6
MMP-12	Matrix metalloproteinase-12	P39900	7.8 (7.3 -- 8.3) [203]	7.9 (7.5 -- 8.5) [12]	1.9
MMP-7	Matrix metalloproteinase-7	P09237	7.3 (6.3 -- 8.1) [203]	7.5 (6.5 -- 8.4) [12]	3.3
NEMO	NF-kappa-B essential modulator	Q9Y6K9	4.2 (3.7 -- 5.0) [203]	4.2 (3.7 -- 5.2) [12]	1.9
PAPPA	Pappalysin-1	Q13219	3.2 (2.9 -- 3.6) [203]	3.3 (2.9 -- 3.6) [12]	1.5
PAR-1	Proteinase-activated receptor 1	P25116	7.4 (7.1 -- 7.7) [203]	7.5 (7.2 -- 7.8) [12]	1.8
PARP-1	Poly [ADP-ribose] polymerase 1	P09874	3.4 (3.0 -- 3.8) [203]	3.5 (3.0 -- 3.9) [12]	1.9
PD-L2	Programmed cell death 1 ligand 2	Q9BQ51	3.1 (2.9 -- 3.3) [203]	3.2 (2.9 -- 3.4) [12]	1.9
PDGF subunit B	Platelet-derived growth factor subunit B	P01127	8.9 (8.1 -- 9.6) [203]	9.0 (8.2 -- 9.6) [12]	1.6
PIgR	Polymeric immunoglobulin receptor	P01833	6.5 (6.4 -- 6.5) [203]	6.5 (6.4 -- 6.5) [12]	1.8
PlGF	Placenta growth factor	P49763	8.1 (7.9 -- 8.3) [203]	8.2 (8.0 -- 8.4) [12]	2.7
PRELP	Prolargin	P51888	6.7 (6.6 -- 6.8) [203]	6.7 (6.6 -- 6.9) [12]	1.5
Protein BOC	Brother of CDO	Q9BWV1	4.9 (4.7 -- 5.0) [203]	4.8 (4.7 -- 5.0) [12]	1.2
PRSS27	Serine protease 27	Q9BQR3	8.1 (7.8 -- 8.4) [203]	8.2 (7.8 -- 8.5) [12]	2.0
PRSS8	Prostasin	Q16651	9.2 (9.0 -- 9.5) [203]	9.3 (9.1 -- 9.5) [12]	1.4
PSGL-1	P-selectin glycoprotein ligand 1	Q14242	5.0 (4.8 -- 5.1) [203]	5.0 (4.8 -- 5.1) [12]	1.3
PTX3	Pentraxin-related protein PTX3	P26022	3.7 (3.4 -- 4.0) [203]	3.7 (3.4 -- 4.0) [12]	1.7
RAGE	Receptor for advanced glycosylation end products	Q15109	5.3 (5.0 -- 5.6) [203]	5.4 (5.1 -- 5.7) [12]	1.1
REN	Renin	P00797	7.5 (6.8 -- 8.1) [203]	7.3 (6.7 -- 8.0) [12]	2.0
SCF	Stem cell factor	P21583	9.7 (9.4 -- 9.9) [203]	9.7 (9.4 -- 9.9) [12]	2.6
SERPINA12	Serpin A12	Q8IW75	3.6 (3.0 -- 4.4) [203]	3.7 (3.1 -- 4.4) [12]	3.0
SLAMF7	SLAM family member 7	Q9NQ25	2.4 (2.4 -- 2.8) [203]	2.4 (2.4 -- 2.9) [12]	2.4
SOD2	Superoxide dismutase [Mn], mitochondrial	P04179	8.9 (8.7 -- 9.0) [203]	8.8 (8.7 -- 9.0) [12]	1.8
SORT1	Sortilin	Q99523	6.4 (6.2 -- 6.5) [203]	6.4 (6.2 -- 6.6) [12]	1.6
SPON2	Spondin-2	Q9BUD6	9.0 (8.9 -- 9.1) [255]	9.0 (8.9 -- 9.1) [18]	2.1
SRC	Proto-oncogene tyrosine-protein kinase Src	P12931	5.5 (4.4 -- 7.0) [203]	5.6 (4.4 -- 6.8) [12]	1.4
STK4	Serine/threonine-protein kinase 4	Q13043	1.9 (1.2 -- 3.5) [203]	1.9 (1.2 -- 3.7) [12]	1.2
TF	Tissue factor	P13726	5.8 (5.6 -- 6.0) [203]	5.8 (5.6 -- 6.1) [12]	1.3

TGM2	Protein-glutamine gamma-glutamyltransferase 2	P21980	8.3 (7.8 -- 8.8) [203]	8.4 (7.9 -- 8.9) [12]	1.9
THBS2	Thrombospondin-2	P35442	6.0 (5.9 -- 6.2) [271]	6.1 (5.9 -- 6.3) [18]	1.1
THPO	Thrombopoietin	P40225	2.1 (1.9 -- 2.3) [203]	2.1 (1.9 -- 2.4) [12]	0.7
TIE2	Angiopoietin-1 receptor	Q02763	8.1 (7.9 -- 8.3) [203]	8.1 (7.9 -- 8.3) [12]	2.0
TM	Thrombomodulin	P07204	8.4 (8.2 -- 8.7) [203]	8.5 (8.2 -- 8.7) [12]	2.3
TIM	T-cell immunoglobulin mucin receptor 1	Q96D42	10.0 (9.5 -- 10.6) [203]	10.2 (9.7 -- 10.8) [12]	3.2
TNFRSF10A	Tumor necrosis factor receptor superfamily member 10A	O00220	3.7 (3.4 -- 3.9) [203]	3.8 (3.5 -- 4.0) [12]	1.7
TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A	Q9Y6Q6	5.8 (5.5 -- 6.2) [203]	5.9 (5.5 -- 6.3) [12]	1.7
TNFRSF13B	Tumor necrosis factor receptor superfamily member 13B	O14836	8.4 (8.1 -- 8.7) [203]	8.5 (8.2 -- 8.8) [12]	2.3
TRAIL-R2	TNF-related apoptosis-inducing ligand receptor 2	O14763	5.8 (5.5 -- 6.1) [203]	5.9 (5.6 -- 6.2) [12]	1.9
VEGF-D	Vascular endothelial growth factor D	O43915	7.4 (7.2 -- 7.7) [203]	7.5 (7.2 -- 7.8) [12]	1.3
VSIG2	V-set and immunoglobulin domain-containing protein 2	Q96IQ7	4.0 (3.6 -- 4.3) [203]	4.1 (3.7 -- 4.4) [12]	1.8
XCL1	Lymphotactin	P47992	5.4 (5.0 -- 5.7) [203]	5.5 (5.1 -- 5.8) [12]	1.4
ALCAM	CD166 antigen	Q13740	4.9 (4.7 -- 5.1) [23]	4.9 (4.7 -- 5.1) [0]	1.7
AP-N	Aminopeptidase N	P15144	5.1 (4.9 -- 5.3) [23]	5.2 (5.0 -- 5.4) [0]	0.8
AXL	Tyrosine-protein kinase receptor UFO	P30530	8.1 (7.9 -- 8.4) [23]	8.2 (8.0 -- 8.4) [0]	1.9
AZU1	Azurocidin	P20160	3.0 (2.5 -- 3.7) [23]	3.1 (2.6 -- 3.7) [0]	2.5
BLM hydrolase	Bleomycin hydrolase	Q13867	5.7 (5.5 -- 6.0) [162]	5.8 (5.5 -- 6.1) [8]	5.4
CASP-3	Caspase-3	P42574	6.5 (5.7 -- 7.8) [23]	6.6 (5.8 -- 7.8) [0]	3.3
CCL15	C-C motif chemokine 15	Q16663	7.3 (7.0 -- 7.7) [23]	7.4 (7.1 -- 7.8) [0]	2.2
CCL16	C-C motif chemokine 16	O15467	6.1 (5.8 -- 6.5) [23]	6.3 (6.0 -- 6.6) [0]	0.4
CCL22	C-C motif chemokine 22	O00175	2.3 (2.0 -- 2.8) [23]	2.4 (2.0 -- 2.7) [0]	1.3
CCL24	C-C motif chemokine 24	O00175	5.8 (5.2 -- 6.4) [23]	5.8 (5.3 -- 6.4) [0]	2.5
CD163	Scavenger receptor cysteine-rich type 1 protein M130	Q86VB7	7.9 (7.6 -- 8.2) [23]	8.0 (7.7 -- 8.3) [0]	2.3
CD93	Complement component C1q receptor	Q9NPY3	9.9 (9.6 -- 10.1) [23]	9.9 (9.7 -- 10.2) [0]	2.3
CDH5	Cadherin-5	Q9NPY3	3.8 (3.5 -- 4.0) [23]	3.8 (3.6 -- 4.1) [0]	1.2

CHI3L1	Chitinase-3-like protein 1	P36222	7.4 (6.8 -- 8.2) [23]	7.6 (6.9 -- 8.4) [0]	3.3
CHIT1	Chitotriosidase-1	Q13231	3.4 (2.6 -- 4.1) [23]	3.5 (2.5 -- 4.4) [0]	-1.2
CNTN1	Contactin-1	Q12860	3.2 (3.0 -- 3.4) [23]	3.2 (3.0 -- 3.4) [0]	0.5
COL1A1	Collagen alpha-1(I) chain	P02452	2.5 (2.2 -- 2.8) [23]	2.5 (2.2 -- 2.8) [0]	-0.1
CPA1	Carboxypeptidase A1	P15085	4.6 (4.2 -- 5.1) [23]	4.6 (4.2 -- 5.1) [0]	0.9
CPB1	Carboxypeptidase B	P15086	4.1 (3.7 -- 4.6) [23]	4.2 (3.8 -- 4.6) [0]	0.6
CSTB	Cystatin-B	P04080	5.1 (4.8 -- 5.6) [93]	5.2 (4.9 -- 5.7) [3]	4.1
CTSD	Cathepsin D	P07339	4.3 (4.0 -- 4.7) [23]	4.4 (4.1 -- 4.8) [0]	3.5
CTSZ	Cathepsin Z	Q9UBR2	5.0 (4.8 -- 5.3) [23]	5.1 (4.8 -- 5.4) [0]	-0.1
CXCL16	C-X-C motif chemokine 16	Q9H2A7	6.3 (6.1 -- 6.5) [23]	6.3 (6.1 -- 6.5) [0]	0.7
DLK-1	Protein delta homolog 1	P80370	5.4 (5.0 -- 5.8) [23]	5.4 (5.0 -- 5.8) [0]	0.7
EGFR	Epidermal growth factor receptor	P00533	1.9 (1.8 -- 2.1) [23]	1.9 (1.8 -- 2.1) [0]	-0.1
Ep-CAM	Epithelial cell adhesion molecule	P16422	4.0 (3.5 -- 4.7) [23]	4.1 (3.6 -- 4.7) [0]	0.9
EPHB4	Ephrin type-B receptor 4	P54760	2.3 (2.1 -- 2.5) [23]	2.3 (2.1 -- 2.5) [0]	1.4
FABP4	Fatty acid-binding protein, adipocyte	P15090	5.1 (4.5 -- 5.7) [23]	5.3 (4.6 -- 5.8) [0]	1.2
FAS	Tumor necrosis factor receptor superfamily member 6	P25445	5.0 (4.8 -- 5.2) [23]	5.1 (4.9 -- 5.3) [0]	1.3
Gal-3	Galectin-3	P17931	5.7 (5.4 -- 5.9) [23]	5.8 (5.5 -- 6.0) [0]	4.0
Gal-4	Galectin-4	P56470	3.8 (3.4 -- 4.2) [23]	3.9 (3.5 -- 4.2) [0]	0.9
GDF-15	Growth/differentiation factor 15	Q99988	5.3 (4.9 -- 5.8) [23]	5.6 (5.2 -- 5.9) [0]	1.9
GRN	Granulins	P28799	3.5 (3.3 -- 3.7) [23]	3.5 (3.4 -- 3.7) [0]	-0.5
ICAM-2	Intercellular adhesion molecule 2	P13598	5.3 (5.0 -- 5.5) [23]	5.3 (5.0 -- 5.6) [0]	1.1
IGFBP-1	Insulin-like growth factor-binding protein 1	P08833	5.4 (4.4 -- 6.2) [23]	5.5 (4.5 -- 6.4) [0]	1.4
IGFBP-2	Insulin-like Growth Factor-Binding Protein 2	P18065	8.1 (7.5 -- 8.5) [23]	8.1 (7.7 -- 8.7) [0]	1.2
IGFBP-7	Insulin-like growth factor-binding protein 7	Q16270	4.6 (4.3 -- 4.9) [23]	4.7 (4.5 -- 5.0) [0]	0.8
IL-17RA	Interleukin-17 receptor A	Q96F46	4.2 (3.8 -- 4.5) [23]	4.2 (3.9 -- 4.5) [0]	1.5
IL-18BP	Interleukin-18-binding protein	O95998	6.7 (6.5 -- 7.0) [23]	6.8 (6.6 -- 7.1) [0]	1.2
IL-1RT1	Interleukin-1 receptor type 1	P14778	6.6 (6.4 -- 6.8) [23]	6.7 (6.4 -- 6.8) [0]	1.7
IL-1RT2	Interleukin-1 receptor type 2	P27930	5.2 (5.0 -- 5.5) [23]	5.2 (5.0 -- 5.4) [0]	1.8
IL-6RA	Interleukin-6 receptor subunit alpha	P08887	11.0 (10.7 -- 11.3) [23]	11.0 (10.7 -- 11.3) [0]	4.0

IL2-RA	Interleukin-2 receptor subunit alpha	P01589	4.2 (3.9 -- 4.6) [23]	4.4 (4.0 -- 4.7) [0]	1.1
ITGB2	Integrin beta-2	P05107	5.7 (5.5 -- 6.0) [23]	5.7 (5.5 -- 6.0) [0]	1.7
JAM-A	Junctional adhesion molecule A	Q9Y624	4.4 (4.1 -- 4.8) [23]	4.5 (4.2 -- 4.9) [0]	1.2
KLK6	Kallikrein-6	Q92876	3.5 (3.5 -- 3.6) [23]	3.5 (3.5 -- 3.7) [0]	3.5
LDL receptor	Low-density lipoprotein receptor	P01130	4.6 (4.2 -- 5.0) [23]	4.7 (4.3 -- 5.0) [0]	0.9
LTBR	Lymphotoxin-beta receptor	P36941	3.9 (3.7 -- 4.1) [23]	4.0 (3.7 -- 4.3) [0]	1.1
MB	Myoglobin	P02144	7.3 (6.9 -- 7.7) [23]	7.3 (6.9 -- 7.7) [0]	1.5
MCP-1	Monocyte chemotactic protein 1	P13500	3.4 (3.2 -- 3.6) [23]	3.4 (3.2 -- 3.6) [0]	0.6
MEPE	Matrix extracellular phosphoglycoprotein	Q9NQ76	3.4 (3.1 -- 3.7) [23]	3.4 (3.1 -- 3.8) [0]	1.6
MMP-2	Matrix metalloproteinase-2	P08253	4.1 (3.9 -- 4.4) [23]	4.2 (4.0 -- 4.4) [0]	0.2
MMP-3	Matrix metalloproteinase-3	P08254	7.5 (7.0 -- 7.9) [23]	7.6 (7.1 -- 8.0) [0]	2.1
MMP-9	Matrix metalloproteinase-9	P14780	4.2 (3.7 -- 4.7) [23]	4.3 (3.7 -- 4.7) [0]	1.6
MPO	Myeloperoxidase	P05164	4.4 (4.1 -- 4.7) [23]	4.4 (4.1 -- 4.7) [0]	2.4
NOTCH-3	Neurogenic locus notch homolog protein 3	Q9UM47	4.4 (4.1 -- 4.7) [23]	4.4 (4.2 -- 4.8) [0]	1.7
NT-proBNP	N-terminal prohormone brain natriuretic peptide	NA	2.8 (2.1 -- 3.4) [23]	3.2 (2.6 -- 3.7) [0]	1.5
OPG	Osteoprotegerin	O00300	3.6 (3.4 -- 3.9) [23]	3.7 (3.5 -- 4.0) [0]	0.8
OPN	Osteopontin	P10451	5.4 (5.0 -- 5.7) [23]	5.6 (5.2 -- 5.9) [0]	0.9
PAI	Plasminogen activator inhibitor 1	P05121	6.2 (5.6 -- 6.9) [23]	6.4 (5.7 -- 6.9) [0]	1.2
PCSK9	Proprotein convertase subtilisin/kexin type 9	Q8NBP7	2.9 (2.6 -- 3.2) [23]	2.9 (2.7 -- 3.1) [0]	1.4
PDGF subunit A	Platelet-derived growth factor subunit A	P04085	3.1 (2.4 -- 3.7) [23]	3.1 (2.5 -- 3.9) [0]	0.0
PECAM-1	Platelet endothelial cell adhesion molecule	P16284	4.8 (4.5 -- 5.0) [23]	4.8 (4.6 -- 5.0) [0]	0.7
PGLYRP1	Peptidoglycan recognition protein 1	O75594	7.7 (7.4 -- 8.0) [23]	7.8 (7.4 -- 8.2) [0]	1.9
PI3	Elafin	P19957	4.2 (3.7 -- 4.6) [89]	4.3 (3.9 -- 4.9) [6]	2.8
PLC	Perlecan	P98160	6.9 (6.7 -- 7.2) [23]	7.0 (6.8 -- 7.3) [0]	3.4
PON3	Paraoxonase	Q15166	5.7 (5.3 -- 6.1) [23]	5.6 (5.2 -- 6.0) [0]	1.2
PRTN3	Myeloblastin	P24158	4.7 (4.4 -- 5.1) [23]	4.7 (4.4 -- 5.2) [0]	3.9
PSP-D	Pulmonary surfactant-associated protein D	P35247	2.8 (2.3 -- 3.3) [23]	2.8 (2.3 -- 3.2) [0]	1.4
RARRES2	Retinoic acid receptor responder protein 2	Q99969	12.1 (11.9 -- 12.2) [23]	12.1 (11.9 -- 12.3) [0]	4.5
RETN	Resistin	Q9HD89	6.9 (6.5 -- 7.2) [23]	7.0 (6.6 -- 7.4) [0]	2.5

SCGB3A2	Secretoglobin family 3A member 2	Q96PL1	2.6 (2.2 -- 3.2) [23]	2.7 (2.3 -- 3.3) [0]	0.4
SELE	E-selectin	P16581	2.5 (2.1 -- 2.8) [23]	2.5 (2.2 -- 2.8) [0]	0.7
SELP	P-selectin	P16109	9.1 (8.7 -- 9.5) [23]	9.1 (8.7 -- 9.6) [0]	2.5
SHPS-1	Tyrosine-protein phosphatase non- receptor type substrate 1	P78324	4.0 (3.7 -- 4.3) [23]	4.0 (3.7 -- 4.3) [0]	1.5
SPON1	Spondin-1	Q9HCB6	2.3 (2.1 -- 2.5) [23]	2.4 (2.2 -- 2.6) [0]	1.4
ST2	ST2 protein	Q01638	4.2 (3.9 -- 4.6) [23]	4.4 (4.0 -- 4.7) [0]	1.5
t-PA	Tissue-type plasminogen activator	P00750	6.3 (5.9 -- 6.7) [23]	6.4 (6.0 -- 6.8) [0]	1.1
TFF3	Trefoil factor 3	Q07654	5.6 (5.3 -- 6.0) [23]	5.7 (5.4 -- 6.2) [0]	2.9
TFPI	Tissue factor pathway inhibitor	P10646	8.6 (8.3 -- 8.8) [23]	8.6 (8.4 -- 8.9) [0]	1.7
TIMP4	Metalloproteinase inhibitor 4	Q99727	5.2 (4.8 -- 5.5) [23]	5.3 (5.0 -- 5.7) [0]	0.7
TLT-2	Trem-like transcript 2 protein	Q5T2D2	4.2 (3.9 -- 4.5) [23]	4.2 (3.9 -- 4.6) [0]	1.5
TNF-R1	Tumor necrosis factor receptor 1	P19438	5.3 (5.0 -- 5.6) [23]	5.4 (5.1 -- 5.8) [0]	1.2
TNF-R2	Tumor necrosis factor receptor 2	P20333	4.9 (4.6 -- 5.2) [23]	5.0 (4.7 -- 5.4) [0]	1.1
TNFRSF10C	Tumor necrosis factor receptor superfamily member 10C	O14798	6.1 (5.8 -- 6.4) [23]	6.0 (5.7 -- 6.4) [0]	1.5
TNFRSF14	Tumor necrosis factor receptor superfamily member 14	Q92956	5.0 (4.8 -- 5.4) [23]	5.1 (4.9 -- 5.5) [0]	1.3
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	Q9Y275	6.5 (6.3 -- 6.8) [23]	6.5 (6.3 -- 6.8) [0]	1.6
TR	Transferrin receptor protein 1	P02786	5.2 (4.8 -- 5.6) [23]	5.3 (4.9 -- 5.7) [0]	1.2
TR-AP	Tartrate-resistant acid phosphatase type 5	P13686	5.0 (4.7 -- 5.3) [23]	5.0 (4.7 -- 5.3) [0]	2.7
U-PAR	Urokinase plasminogen activator surface receptor	Q03405	4.8 (4.5 -- 5.1) [23]	4.9 (4.6 -- 5.2) [0]	1.9
uPA	Urokinase-type plasminogen activator	P00749	5.0 (4.8 -- 5.2) [23]	5.0 (4.8 -- 5.3) [0]	1.0
vWF	von Willebrand factor	P04275	6.4 (5.8 -- 7.0) [23]	6.6 (6.1 -- 7.3) [0]	1.4
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1	Q13541	8.4 (7.8 -- 9.1) [188]	8.5 (8.0 -- 9.3) [18]	1.4
ADA	Adenosine Deaminase	P00813	4.1 (3.9 -- 4.4) [188]	4.1 (3.9 -- 4.4) [18]	0.3
ARTN	Artemin	Q5T4W7	0.2 (0.2 -- 0.2) [188]	0.2 (0.2 -- 0.2) [18]	0.2
AXIN1	Axin-1	O15169	1.6 (1.4 -- 2.5) [188]	1.6 (1.4 -- 2.7) [18]	1.4
BDNF	Brain-derived neurotrophic factor	P23560	2.4 (2.4 -- 6.2) [188]	2.4 (2.4 -- 5.9) [18]	2.4

Beta-NGF	Beta-nerve growth factor	P01138	1.6 (1.4 -- 1.8) [188]	1.6 (1.5 -- 1.8) [18]	0.8
CASP-8	Caspase 8	Q14790	2.8 (2.4 -- 3.5) [188]	3.0 (2.4 -- 3.7) [18]	1.0
CCL11	Eotaxin-1	P51671	8.1 (7.9 -- 8.4) [188]	8.2 (7.9 -- 8.5) [18]	1.3
CCL19	C-C motif chemokine 19	Q99731	9.4 (9.0 -- 10.0) [188]	9.6 (9.0 -- 10.1) [18]	1.4
CCL20	C-C motif chemokine 20	P78556	6.6 (6.1 -- 7.3) [188]	6.8 (6.1 -- 7.5) [18]	1.8
CCL23	C-C motif chemokine 23	P55773	10.0 (9.7 -- 10.4) [188]	10.1 (9.9 -- 10.4) [18]	1.1
CCL25	C-C motif chemokine 25	O15444	6.9 (6.5 -- 7.3) [188]	6.9 (6.5 -- 7.3) [18]	1.0
CCL28	C-C motif chemokine 28	Q9NRJ3	1.0 (0.8 -- 1.2) [188]	1.1 (0.9 -- 1.3) [18]	0.1
CCL4	C-C motif chemokine 4	P13236	5.8 (5.5 -- 6.2) [188]	5.9 (5.5 -- 6.3) [18]	0.3
CD244	Natural killer cell receptor 2B4	Q9BZW8	6.0 (5.8 -- 6.2) [188]	6.0 (5.8 -- 6.3) [18]	1.7
CD40	CD40L receptor	P25942	9.3 (9.1 -- 9.6) [188]	9.4 (9.2 -- 9.7) [18]	1.3
CD5	T-cell surface glycoprotein CD5	P06127	3.4 (3.2 -- 3.7) [188]	3.5 (3.3 -- 3.8) [18]	-0.4
CD6	T cell surface glycoprotein CD6 isoform	Q8WWJ7	4.0 (3.7 -- 4.2) [188]	4.0 (3.7 -- 4.3) [18]	0.8
CDCP1	CUB domain-containing protein 1	Q9H5V8	3.0 (2.6 -- 3.4) [188]	3.1 (2.7 -- 3.6) [18]	0.0
CSF-1	Macrophage colony-stimulating factor 1	P09603	8.0 (7.8 -- 8.1) [188]	8.0 (7.9 -- 8.1) [18]	0.6
CST5	Cystatin D	P28325	7.0 (6.6 -- 7.4) [188]	7.0 (6.6 -- 7.4) [18]	3.2
CX3CL1	Fractalkine	P78423	5.8 (5.5 -- 6.0) [188]	5.8 (5.5 -- 6.2) [18]	1.6
CXCL10	C-X-C motif chemokine 10	P02778	10.3 (9.8 -- 10.8) [188]	10.4 (10.0 -- 11.0) [18]	2.0
CXCL11	C-X-C motif chemokine 11	O14625	6.9 (6.4 -- 7.5) [188]	7.0 (6.4 -- 7.7) [18]	1.7
CXCL5	C-X-C motif chemokine 5	P42830	10.7 (9.5 -- 11.7) [188]	10.8 (9.6 -- 11.8) [18]	3.7
CXCL6	C-X-C motif chemokine 6	P80162	7.3 (6.8 -- 7.9) [188]	7.4 (6.9 -- 7.9) [18]	1.3
CXCL9	C-X-C motif chemokine 9	Q07325	8.5 (8.0 -- 9.1) [188]	8.6 (8.1 -- 9.2) [18]	1.9
DNER	Delta and Notch-like epidermal growth factor-related receptor	Q8NFT8	7.1 (6.9 -- 7.3) [188]	7.1 (6.9 -- 7.3) [18]	0.6
EN-RAGE	Protein S100-A12	P80511	2.7 (2.1 -- 3.2) [188]	2.8 (2.3 -- 3.3) [18]	0.7
FGF-19	Fibroblast growth factor 19	O95750	8.1 (7.4 -- 8.8) [188]	8.2 (7.4 -- 8.9) [18]	1.1
FGF-5	Fibroblast growth factor 5	Q8NF90	1.9 (1.7 -- 2.1) [188]	1.9 (1.8 -- 2.1) [18]	1.4
Flt3L	Fms-related tyrosine kinase 3 ligand	P49771	9.3 (9.1 -- 9.6) [188]	9.4 (9.1 -- 9.6) [18]	1.8
hGDNF	Glial cell line-derived neurotrophic factor	P39905	2.2 (2.0 -- 2.5) [188]	2.3 (2.0 -- 2.5) [18]	1.5

HGF	Hepatocyte growth factor	P14210	7.5 (7.2 -- 7.8) [188]	7.6 (7.4 -- 7.9) [18]	0.8
IFN-gamma	Interferon gamma	P01579	1.1 (1.1 -- 1.1) [188]	1.1 (1.1 -- 1.1) [18]	1.1
IL-1 alpha	Interleukin-1 alpha	P01583	1.7 (1.7 -- 1.7) [188]	1.7 (1.7 -- 1.7) [18]	1.7
IL-10	Interleukin-10	P22301	4.3 (4.0 -- 4.6) [188]	4.3 (4.0 -- 4.6) [18]	2.1
IL-10RA	Interleukin-10 receptor subunit alpha	Q13651	0.9 (0.9 -- 0.9) [188]	0.9 (0.9 -- 0.9) [18]	0.9
IL-10RB	Interleukin-10 receptor subunit beta	Q08334	6.7 (6.5 -- 6.9) [188]	6.7 (6.5 -- 7.0) [18]	0.8
IL-12B	Interleukin-12 subunit beta	P29460	4.8 (4.4 -- 5.3) [188]	5.0 (4.5 -- 5.4) [18]	0.9
IL-13	Interleukin-13	P35225	1.1 (1.1 -- 1.1) [188]	1.1 (1.1 -- 1.1) [18]	1.1
IL-15RA	Interleukin-15 receptor subunit alpha	Q13261	1.1 (0.9 -- 1.3) [188]	1.1 (0.9 -- 1.3) [18]	0.5
IL-17A	Interleukin-17A	Q16552	0.5 (0.4 -- 0.8) [188]	0.5 (0.4 -- 0.8) [18]	0.4
IL-17C	Interleukin-17C	Q9P0M4	1.5 (1.5 -- 1.8) [188]	1.6 (1.5 -- 1.8) [18]	1.5
IL-18R1	Interleukin-18 receptor 1	Q13478	7.5 (7.2 -- 7.8) [188]	7.6 (7.3 -- 7.8) [18]	0.8
IL-2	Interleukin-2	P60568	1.4 (1.4 -- 1.4) [188]	1.4 (1.4 -- 1.4) [18]	1.4
IL-20	Interleukin-20	Q9NYY1	0.8 (0.8 -- 0.8) [188]	0.8 (0.8 -- 0.8) [18]	0.8
IL-20RA	Interleukin-20 receptor subunit alpha	Q9UHF4	0.9 (0.9 -- 0.9) [188]	0.9 (0.9 -- 0.9) [18]	0.9
IL-22 RA1	Interleukin-22 receptor subunit alpha-1	Q8N6P7	2.3 (2.3 -- 2.3) [188]	2.3 (2.3 -- 2.3) [18]	2.3
IL-24	Interleukin-24	Q13007	0.4 (0.4 -- 0.4) [188]	0.4 (0.4 -- 0.4) [18]	0.4
IL-2RB	Interleukin-2 receptor subunit beta	P14784	0.8 (0.8 -- 0.8) [188]	0.8 (0.8 -- 0.8) [18]	0.8
IL-33	Interleukin-33	O95760	1.7 (1.7 -- 1.7) [188]	1.7 (1.7 -- 1.7) [18]	1.7
IL-4	Interleukin-4	P05112	1.5 (1.5 -- 1.5) [188]	1.5 (1.5 -- 1.5) [18]	1.5
IL-5	Interleukin-5	P05113	1.6 (1.6 -- 1.6) [188]	1.6 (1.6 -- 1.6) [18]	1.6
IL-7	Interleukin-7	P13232	3.3 (2.8 -- 3.8) [254]	3.3 (2.8 -- 3.9) [22]	1.5
IL-8	Interleukin-8	P10145	6.3 (5.9 -- 6.7) [188]	6.4 (6.1 -- 7.0) [18]	5.6
LAP TGF-beta-1	Latency-associated peptide transforming growth factor beta 1	P01137	6.3 (6.1 -- 6.6) [188]	6.4 (6.1 -- 6.7) [18]	0.8
LIF	Leukemia inhibitory factor	P15018	0.6 (0.6 -- 0.6) [188]	0.6 (0.6 -- 0.6) [18]	0.6
LIF-R	Leukemia inhibitory factor receptor	P42702	4.0 (3.8 -- 4.1) [188]	4.0 (3.8 -- 4.2) [18]	2.3
MCP-2	Monocyte chemotactic protein 2	P80075	9.0 (8.6 -- 9.4) [188]	9.1 (8.6 -- 9.5) [18]	5.1
MCP-3	Monocyte chemotactic protein 3	P80098	2.1 (1.9 -- 2.4) [188]	2.2 (1.9 -- 2.5) [18]	1.9

MCP-4	Monocyte chemotactic protein 4	Q99616	2.3 (1.9 -- 2.7) [188]	2.3 (2.0 -- 2.8) [18]	0.3
MMP-1	Matrix metalloproteinase-1	P03956	7.5 (6.8 -- 8.3) [188]	7.6 (6.9 -- 8.4) [18]	-0.3
MMP-10	Matrix metalloproteinase-10	P09238	9.3 (8.9 -- 9.7) [188]	9.3 (8.8 -- 9.7) [18]	2.1
NRTN	Neurturin	Q99748	1.2 (1.2 -- 1.2) [188]	1.2 (1.2 -- 1.2) [18]	1.2
NT-3	Neurotrophin-3	P20783	1.9 (1.7 -- 2.2) [188]	2.0 (1.7 -- 2.2) [18]	0.6
OSM	Oncostatin-M	P13725	2.6 (2.1 -- 3.1) [188]	2.7 (2.2 -- 3.2) [18]	0.5
PD-L1	Programmed cell death 1 ligand 1	Q9NZQ7	5.0 (4.8 -- 5.3) [188]	5.1 (4.8 -- 5.4) [18]	2.4
SIRT2	SIR2-like protein 2	Q8IXJ6	4.1 (3.4 -- 5.3) [188]	4.2 (3.6 -- 5.4) [18]	2.0
SLAMF1	Signaling lymphocytic activation molecule	Q13291	3.5 (3.2 -- 3.9) [188]	3.5 (3.2 -- 3.9) [18]	1.8
ST1A1	Sulfotransferase 1A1	P50225	1.3 (0.5 -- 2.7) [188]	1.3 (0.5 -- 2.8) [18]	0.3
STAMPB	STAM-binding protein	O95630	3.3 (2.9 -- 4.1) [188]	3.4 (3.0 -- 4.2) [18]	1.3
TGF-alpha	Transforming growth factor alpha	P01135	1.2 (1.0 -- 1.4) [188]	1.3 (1.0 -- 1.5) [18]	-1.0
TNF	Tumor necrosis factor	P01375	0.9 (0.9 -- 0.9) [188]	0.9 (0.9 -- 0.9) [18]	0.9
TNFB	TNF-beta	P01374	3.1 (2.8 -- 3.3) [188]	3.1 (2.8 -- 3.3) [18]	0.6
TNFRSF9	Tumor necrosis factor receptor superfamily member 9	Q07011	6.5 (6.1 -- 6.8) [188]	6.6 (6.2 -- 6.9) [18]	1.7
TNFSF14	Tumor necrosis factor ligand superfamily member 14	O43557	2.6 (2.3 -- 3.0) [188]	2.7 (2.4 -- 3.0) [18]	-0.0
TRAIL	TNF-related apoptosis-inducing ligand	P50591	7.6 (7.4 -- 7.8) [188]	7.6 (7.4 -- 7.8) [18]	6.4
TRANCE	TNF-related activation-induced cytokine	O14788	4.6 (4.2 -- 5.0) [188]	4.6 (4.1 -- 5.0) [18]	1.4
TSLP	Thymic stromal lymphopoietin	Q969D9	1.1 (1.1 -- 1.1) [188]	1.1 (1.1 -- 1.1) [18]	1.1
TWEAK	Tumor necrosis factor (Ligand) superfamily, member 12	Q4ACW9	8.3 (8.1 -- 8.5) [188]	8.3 (8.0 -- 8.5) [18]	0.7
VEGF-A	Vascular endothelial growth factor A	P15692	10.1 (9.9 -- 10.4) [188]	10.2 (10.0 -- 10.5) [18]	2.3

Continuous variables presented as median (Q1-Q3). Number of missing values presented in [n]. SE, systemic embolism.
The CVDII panel were used for biomarkers ranging from ACE2 to XCL1; CVDIII panel for ALCAM to vWF; and Inflammation panel for 4E-BP1 to VEGF-A.

Table S2. Baseline NPX values (arbitrary units) and limit of detection (LoD) of proximity extension assay (PEA) biomarkers in the validation cohort.

Variable		UniProt No.	No	Ischemic stroke/SE	LoD
ACE2	Angiotensin-converting enzyme 2	Q9BYF1	4.4 (3.9 -- 4.8)	4.4 (4.1 -- 5.0)	0.3
ADAM-TS13	A disintegrin and metalloproteinase with thrombospondin motifs 13	Q76LX8	5.9 (5.8 -- 6.0)	5.9 (5.8 -- 6.0)	1.1
ADM	ADM	P35318	7.3 (6.9 -- 7.6)	7.3 (7.0 -- 7.7)	1.2
AGRP	Agouti-related protein	O00253	5.0 (4.7 -- 5.3)	5.1 (4.8 -- 5.3)	0.8
AMBP	Protein AMBP	P02760	7.5 (7.3 -- 7.6)	7.5 (7.3 -- 7.6)	0.8
ANGPT1	Angiopoietin-1	Q15389	8.0 (7.1 -- 8.8)	8.2 (7.2 -- 9.1)	0.6
BMP-6	Bone morphogenetic protein 6	P22004	4.8 (4.5 -- 5.1)	4.9 (4.6 -- 5.2)	0.8
BNP	Natriuretic peptides B	P16860	5.4 (4.0 -- 6.4)	5.7 (4.4 -- 6.8)	1.5
Protein BOC	Brother of CDO	Q9BWV1	3.9 (3.7 -- 4.1)	3.9 (3.7 -- 4.2)	1.0
CA5A	Carbonic anhydrase 5A, mitochondrial	Q92583	2.2 (1.7 -- 2.9)	2.3 (1.8 -- 3.0)	1.6
CCL17	C-C motif chemokine 17	P10147	7.7 (7.1 -- 8.5)	7.8 (7.4 -- 8.7)	1.3
CCL3	C-C motif chemokine 3	P01730	6.3 (5.9 -- 6.7)	6.4 (6.1 -- 6.9)	1.2
CD4	T-cell surface glycoprotein CD4	P29965	5.3 (5.1 -- 5.5)	5.4 (5.2 -- 5.6)	0.7
CD40-L	CD40 ligand	Q9UIB8	3.6 (3.0 -- 4.6)	3.9 (3.3 -- 5.2)	0.8
CD84	SLAM family member 5	P31997	4.1 (3.8 -- 4.4)	4.2 (3.9 -- 4.4)	1.6
CEACAM8	Carcinoembryonic antigenrelated cell adhesion molecule 8	Q99895	4.3 (3.9 -- 4.7)	4.3 (4.0 -- 4.7)	1.7
CTRC	Chymotrypsin C	P07711	10.2 (9.7 -- 10.7)	10.2 (9.8 -- 10.8)	1.9
CTSL1	Cathepsin L1	P09341	6.9 (6.7 -- 7.1)	7.0 (6.7 -- 7.3)	0.9
CXCL1	C-X-C motif chemokine 1	P07585	8.8 (7.9 -- 9.6)	9.0 (8.0 -- 9.7)	2.1
DCN	Decorin	Q16698	4.9 (4.7 -- 5.1)	5.0 (4.8 -- 5.2)	0.9
DECR1	2,4-dienoyl-CoA reductase, mitochondrial	O94907	3.7 (3.1 -- 4.6)	3.9 (3.2 -- 4.7)	1.7
Dkk-1	Dickkopf-related protein 1	P12104	8.4 (8.1 -- 8.9)	8.6 (8.3 -- 9.1)	0.8
FABP2	Fatty acid-binding protein, intestinal	Q9NSA1	8.8 (8.2 -- 9.4)	8.8 (8.3 -- 9.4)	1.5

FGF-21	Fibroblast growth factor 21	Q9GZV9	7.9 (7.0 -- 8.8)	7.7 (7.1 -- 8.6)	1.8
FGF-23	Fibroblast growth factor 23	P19883	4.7 (4.3 -- 5.2)	5.1 (4.5 -- 5.7)	2.0
FS	Follistatin	O00182	11.4 (11.1 -- 11.7)	11.4 (11.2 -- 11.7)	2.0
GAL-9	Galectin-9	Q9UK05	7.8 (7.5 -- 8.0)	7.8 (7.6 -- 8.1)	1.2
GDF-2	Growth/differentiation factor 2	P01241	9.0 (8.6 -- 9.3)	9.1 (8.7 -- 9.3)	1.4
GH	Growth hormone	P27352	7.5 (5.9 -- 8.9)	7.7 (6.3 -- 9.1)	1.1
GIF	Gastric intrinsic factor	Q04760	7.7 (7.1 -- 8.4)	7.8 (7.1 -- 8.3)	1.4
GLO1	Lactoylglutathione lyase	P51161	4.3 (3.8 -- 4.9)	4.4 (3.9 -- 4.9)	1.1
GT	Gastrotropin	Q9UJM8	1.9 (1.5 -- 2.4)	1.8 (1.5 -- 2.3)	0.5
HAOX1	Hydroxyacid oxidase 1	Q99075	5.4 (4.4 -- 6.4)	5.3 (4.4 -- 6.8)	1.0
HB-EGF	Proheparin-binding EGF-like growth factor	P09601	5.3 (5.0 -- 5.7)	5.4 (5.1 -- 6.0)	0.8
HO-1	Heme oxygenase 1	Q8IYS5	11.4 (11.1 -- 11.6)	11.4 (11.2 -- 11.6)	1.7
hOSCAR	Osteoclast-associated immunoglobulin-like receptor	P04792	10.4 (10.2 -- 10.5)	10.4 (10.3 -- 10.6)	1.8
HSP-27	Heat shock 27 kDa protein	P35475	9.1 (8.5 -- 9.6)	9.2 (8.7 -- 9.6)	2.5
IDUA	Alpha-L-iduronidase	P31994	5.4 (5.0 -- 5.7)	5.5 (5.2 -- 5.7)	-0.5
Ig G Fc receptor II-b	Low affinity immunoglobulin gamma Fc region receptor II-b	Q8TAD2	3.2 (2.5 -- 3.8)	3.4 (2.9 -- 4.0)	1.1
IL-17D	Interleukin-17D	Q14116	2.7 (2.4 -- 2.8)	2.7 (2.4 -- 2.9)	1.4
IL-1ra	Interleukin-1 receptor antagonist protein	P18510	4.6 (4.2 -- 5.1)	4.7 (4.3 -- 5.2)	1.0
IL-27	Interleukin-27	Q8NEV9, Q14213	5.8 (5.5 -- 6.1)	5.8 (5.4 -- 6.2)	1.1
IL-4RA	Interleukin-4 receptor subunit alpha	P24394	1.9 (1.6 -- 2.1)	1.9 (1.7 -- 2.2)	1.1
IL16	Pro-interleukin-16	Q14005	6.4 (6.1 -- 6.7)	6.4 (6.1 -- 6.8)	-0.2
IL-18	Interleukin-18	Q14116	8.5 (8.2 -- 8.9)	8.6 (8.4 -- 9.0)	1.1
IL1RL2	Interleukin-1 receptor-like 2	Q9HB29	4.5 (4.2 -- 4.7)	4.5 (4.2 -- 4.8)	1.3
IL6	Interleukin-6	P05231	3.5 (3.0 -- 4.1)	3.7 (3.2 -- 4.2)	1.2
ITGB1BP2	Melusin	Q9UKP3	2.7 (2.5 -- 4.2)	2.8 (2.5 -- 4.2)	2.5
LEP	Leptin	P41159	7.0 (6.3 -- 7.7)	7.0 (6.2 -- 7.7)	1.4
LOX-1	Lectin-like oxidized LDL receptor 1	P78380	6.6 (6.2 -- 7.0)	6.7 (6.4 -- 7.0)	1.4

LPL	Lipoprotein lipase	P06858	9.5 (9.1 -- 9.8)	9.5 (9.1 -- 9.8)	2.2
MARCO	Macrophage receptor MARCO	Q9UEW3	6.3 (6.1 -- 6.4)	6.3 (6.1 -- 6.4)	1.4
MERTK	Tyrosine-protein kinase Mer	Q12866	6.0 (5.7 -- 6.2)	6.0 (5.7 -- 6.3)	1.4
MMP-12	Matrix metalloproteinase-12	P39900	7.0 (6.5 -- 7.5)	7.3 (6.8 -- 7.9)	0.1
MMP-7	Matrix metalloproteinase-7	P09237	10.2 (9.8 -- 10.5)	10.3 (9.8 -- 10.7)	3.0
NEMO	NF-kappa-B essential modulator	Q9Y6K9	3.2 (2.7 -- 4.0)	3.3 (2.8 -- 4.0)	1.5
PAPPA	Pappalysin-1	Q13219	3.4 (3.0 -- 3.8)	3.5 (3.1 -- 3.9)	0.8
PAR-1	Proteinase-activated receptor 1	P25116	8.6 (8.3 -- 8.9)	8.7 (8.4 -- 9.0)	1.2
PARP-1	Poly [ADP-ribose] polymerase 1	P09874	3.0 (2.6 -- 3.3)	3.0 (2.6 -- 3.4)	1.4
PD-L2	Programmed cell death 1 ligand 2	Q9BQ51	3.1 (2.9 -- 3.3)	3.2 (3.0 -- 3.5)	0.9
PDGF subunit B	Platelet-derived growth factor subunit B	P01127	8.7 (7.8 -- 9.6)	9.0 (8.0 -- 9.8)	1.9
PlGF	Placenta growth factor (PlGF)	P49763	7.9 (7.7 -- 8.2)	8.0 (7.8 -- 8.2)	1.1
PIgR	Polymeric immunoglobulin receptor	P01833	6.3 (6.2 -- 6.4)	6.3 (6.2 -- 6.4)	2.3
PRELP	Prolargin	P51888	8.1 (8.0 -- 8.3) [1]	8.2 (8.0 -- 8.3) [0]	0.8
PRSS27	Serine protease 27	Q9BQR3	8.3 (8.0 -- 8.6)	8.4 (8.1 -- 8.8)	0.9
PRSS8	Prostasin	Q16651	8.5 (8.2 -- 8.7)	8.5 (8.3 -- 8.7)	0.1
PSGL-1	P-selectin glycoprotein ligand 1	Q14242	3.8 (3.7 -- 4.0)	3.9 (3.7 -- 4.0)	0.7
PTX3	Pentraxin-related protein PTX3	P26022	4.3 (4.0 -- 4.7)	4.4 (4.1 -- 4.7)	1.1
RAGE	Receptor for advanced glycosylation end products	Q15109	13.3 (13.0 -- 13.6)	13.5 (13.0 -- 13.8)	2.3
REN	Renin	P00797	6.9 (6.2 -- 7.6)	7.0 (6.4 -- 7.7)	1.3
SCF	Stem cell factor	P21583	8.7 (8.4 -- 9.0)	8.7 (8.4 -- 9.0)	1.2
SERPINA12	Serpin A12	Q8IW75	2.5 (2.0 -- 3.2)	2.6 (2.0 -- 3.2)	0.6
SLAMF7	SLAM family member 7	Q9NQ25	4.0 (3.6 -- 4.5) [1]	4.0 (3.7 -- 4.4) [0]	2.1
SOD2	Superoxide dismutase [Mn], mitochondrial	P04179	9.4 (9.4 -- 9.5)	9.4 (9.4 -- 9.5)	1.3
SORT1	Sortilin	Q99523	8.5 (8.3 -- 8.7)	8.6 (8.4 -- 8.8)	1.2
SPON2	Spondin-2	Q9BUD6	8.2 (8.0 -- 8.3)	8.2 (8.1 -- 8.3)	0.6
SRC	Proto-oncogene tyrosine-protein kinase Src	P12931	5.1 (3.9 -- 6.8)	5.1 (4.1 -- 6.8)	0.6
STK4	Serine/threonine-protein kinase 4	Q13043	2.4 (1.3 -- 4.2)	2.5 (1.3 -- 4.0)	1.3
TF	Tissue factor	P13726	5.2 (5.0 -- 5.4)	5.2 (5.1 -- 5.4)	0.7

TGM2	Protein-glutamine gamma-glutamyltransferase 2	P21980	8.7 (8.3 -- 9.2)	8.8 (8.4 -- 9.2)	2.5
THBS2	Thrombospondin-2	P35442	5.6 (5.4 -- 5.8)	5.6 (5.5 -- 5.8)	0.2
THPO	Thrombopoietin	P40225	2.7 (2.5 -- 2.9)	2.7 (2.5 -- 3.0)	0.6
TIE2	Angiopoietin-1 receptor	Q02763	7.2 (7.0 -- 7.3)	7.2 (7.0 -- 7.4)	1.4
TM	Thrombomodulin	P07204	10.3 (10.0 -- 10.5)	10.3 (10.1 -- 10.6)	2.3
TIM	T-cell immunoglobulin mucin receptor 1	Q96D42	7.4 (6.9 -- 7.9)	7.7 (7.3 -- 8.4)	1.7
TNFRSF10A	Tumor necrosis factor receptor superfamily member 10A	O00220	3.8 (3.5 -- 4.0)	3.9 (3.6 -- 4.1)	1.4
TNFRSF11A	Tumor necrosis factor receptor superfamily member 11A	Q9Y6Q6	5.8 (5.5 -- 6.1)	5.9 (5.6 -- 6.2)	1.3
TNFRSF13B	Tumor necrosis factor receptor superfamily member 13B	O14836	10.1 (9.9 -- 10.4)	10.2 (9.8 -- 10.5)	1.4
TRAIL.R2	TNF-related apoptosis-inducing ligand receptor 2	O14763	5.9 (5.7 -- 6.2)	6.0 (5.9 -- 6.4)	1.5
VEGFD	Vascular endothelial growth factor D	O43915	7.6 (7.4 -- 7.9)	7.7 (7.4 -- 8.0)	0.7
VSIG2	V-set and immunoglobulin domain-containing protein 2	Q96IQ7	4.8 (4.5 -- 5.2)	4.9 (4.6 -- 5.3)	1.6
XCL1	Lymphotactin	P47992	5.0 (4.6 -- 5.4)	5.0 (4.6 -- 5.4)	0.3
ALCAM	CD166 antigen	Q13740	7.2 (7.0 -- 7.3)	7.2 (7.0 -- 7.4)	1.2
AP-N	Aminopeptidase N	P15144	4.7 (4.5 -- 4.9)	4.7 (4.6 -- 4.9)	1.1
AXL	Tyrosine-protein kinase receptor UFO	P30530	8.8 (8.6 -- 9.1)	8.9 (8.7 -- 9.1)	2.4
AZU1	Azurocidin	P20160	2.5 (2.1 -- 3.1)	2.5 (2.1 -- 3.1)	0.6
BLM hydrolase	Bleomycin hydrolase	Q13867	2.1 (1.9 -- 2.4)	2.1 (1.9 -- 2.4)	-0.6
CASP-3	Caspase-3	P42574	4.7 (3.9 -- 6.1)	4.7 (4.0 -- 6.0)	0.7
CCL15	C-C motif chemokine 15	Q16663	7.1 (6.8 -- 7.5)	7.2 (6.9 -- 7.5)	1.2
CCL16	C-C motif chemokine 16	O15467	6.7 (6.4 -- 7.0)	6.8 (6.4 -- 7.1)	0.4
CCL24	C-C motif chemokine 24	O00175	5.0 (4.4 -- 5.7)	4.9 (4.3 -- 5.6)	0.8
CD163	Scavenger receptor cysteine-rich type 1 protein M130	Q86VB7	7.7 (7.4 -- 8.0)	7.8 (7.5 -- 8.0)	1.2
CD93	Complement component C1q receptor	Q9NPY3	10.9 (10.6 -- 11.1)	11.0 (10.7 -- 11.2)	1.7
CDH5	Cadherin-5	Q9NPY3	4.3 (4.1 -- 4.5)	4.3 (4.1 -- 4.5)	1.4
CHI3L1	Chitinase-3-like protein 1	P36222	4.6 (3.9 -- 5.3)	4.8 (4.2 -- 5.4)	-0.8
CHIT1	Chitotriosidase-1	Q13231	5.3 (4.6 -- 6.1)	5.5 (4.7 -- 6.3)	0.8
CNTN1	Contactin-1	Q12860	4.3 (4.1 -- 4.6)	4.4 (4.1 -- 4.6)	0.7
COL1A1	Collagen alpha-1(I) chain	P02452	2.7 (2.5 -- 2.9)	2.8 (2.5 -- 3.0)	0.5

CPA1	Carboxypeptidase A1	P15085	5.7 (5.3 -- 6.2)	5.8 (5.3 -- 6.2)	1.3
CPB1	Carboxypeptidase B	P15086	5.6 (5.1 -- 6.0)	5.6 (5.1 -- 6.0)	0.6
CSTB	Cystatin-B	P04080	4.0 (3.7 -- 4.4)	4.1 (3.9 -- 4.5)	1.9
CTSD	Cathepsin D	P07339	2.4 (2.1 -- 2.7)	2.4 (2.1 -- 2.8)	0.1
CTSZ	Cathepsin Z	Q9UBR2	5.1 (4.9 -- 5.4)	5.2 (5.0 -- 5.4)	1.0
CXCL16	C-X-C motif chemokine 16	Q9H2A7	5.2 (5.1 -- 5.4)	5.3 (5.1 -- 5.4)	1.4
DLK.1	Protein delta homolog 1	P80370	5.8 (5.3 -- 6.1)	5.8 (5.3 -- 6.2)	1.4
EGFR	Epidermal growth factor receptor	P00533	2.8 (2.6 -- 2.9)	2.7 (2.6 -- 2.9)	0.3
EP.CAM	Epithelial cell adhesion molecule	P16422	5.1 (4.5 -- 5.8)	5.0 (4.5 -- 5.7)	2.0
EPHB4	Ephrin type-B receptor 4	P54760	5.5 (5.2 -- 5.7)	5.5 (5.3 -- 5.8)	1.6
FABP4	Fatty acid-binding protein, adipocyte	P15090	5.7 (5.1 -- 6.3)	5.8 (5.3 -- 6.4)	2.0
FAS	Tumor necrosis factor receptor superfamily member 6	P25445	5.8 (5.6 -- 6.0) [0]	5.8 (5.6 -- 6.1) [1]	0.6
Gal-3	Galectin-3	P17931	3.2 (2.9 -- 3.4)	3.2 (3.0 -- 3.4)	-1.1
Gal-4	Galectin-4	P56470	4.1 (3.8 -- 4.5)	4.2 (3.9 -- 4.6)	1.0
GDF-15	Growth/differentiation factor 15	Q99988	6.2 (5.9 -- 6.7)	6.5 (6.1 -- 7.0)	0.6
GP6	Platelet glycoprotein VI	Q9HCN6	1.9 (1.6 -- 2.4)	2.0 (1.7 -- 2.4)	1.1
GRN	Granulins	P28799	5.4 (5.2 -- 5.6)	5.5 (5.3 -- 5.7)	0.4
ICAM-2	Intercellular adhesion molecule 2	P13598	5.4 (5.1 -- 5.6)	5.5 (5.2 -- 5.7)	1.6
IGFBP-1	Insulin-like growth factor-binding protein 1	P08833	4.8 (3.9 -- 5.6)	4.8 (4.2 -- 5.7)	1.6
IGFBP-2	Insulin-like Growth Factor-Binding Protein 2	P18065	8.2 (7.6 -- 8.6)	8.4 (8.0 -- 8.7)	0.8
IGFBP-7	Insulin-like growth factor-binding protein 7	Q16270	7.9 (7.6 -- 8.1)	8.0 (7.7 -- 8.3)	1.4
IL-17RA	Interleukin-17 receptor A	Q96F46	4.0 (3.7 -- 4.3)	4.1 (3.7 -- 4.3)	1.6
IL-18BP	Interleukin-18-binding protein	O95998	5.8 (5.6 -- 6.1)	5.9 (5.7 -- 6.2)	1.3
IL-1RT1	Interleukin-1 receptor type 1	P14778	6.3 (6.2 -- 6.5)	6.4 (6.2 -- 6.7)	2.1
IL-1RT2	Interleukin-1 receptor type 2	P27930	4.9 (4.7 -- 5.1)	4.8 (4.6 -- 5.1)	0.8
IL-6RA	Interleukin-6 receptor subunit alpha	P08887	11.6 (11.3 -- 11.9)	11.6 (11.3 -- 11.8)	2.7
IL2-RA	Interleukin-2 receptor subunit alpha	P01589	3.8 (3.5 -- 4.1)	3.9 (3.6 -- 4.1)	1.8
ITGB2	Integrin beta-2	P05107	5.4 (5.1 -- 5.6)	5.4 (5.2 -- 5.7)	1.0
JAM-A	Junctional adhesion molecule A	Q9Y624	3.8 (3.5 -- 4.1)	3.9 (3.7 -- 4.3)	0.2

KLK6	Kallikrein-6	Q92876	2.4 (2.1 -- 2.6)	2.4 (2.2 -- 2.7)	0.3
LDL receptor	Low-density lipoprotein receptor	P01130	3.7 (3.4 -- 4.1)	3.7 (3.3 -- 4.1)	0.2
LTBR	Lymphotoxin-beta receptor	P36941	3.9 (3.7 -- 4.2)	4.0 (3.8 -- 4.3)	1.5
MB	Myoglobin	P02144	7.7 (7.3 -- 8.1)	7.7 (7.3 -- 8.0)	2.4
MCP-1	Monocyte chemotactic protein 1	P13500	4.2 (3.9 -- 4.4)	4.2 (4.0 -- 4.5)	1.1
MEPE	Matrix extracellular phosphoglycoprotein	Q9NQ76	5.6 (5.3 -- 5.9)	5.6 (5.3 -- 5.9)	1.3
MMP-2	Matrix metalloproteinase-2	P08253	3.7 (3.5 -- 3.9)	3.7 (3.5 -- 4.0)	2.5
MMP-3	Matrix metalloproteinase-3	P08254	7.7 (7.2 -- 8.1)	7.7 (7.4 -- 8.1)	1.7
MMP-9	Matrix metalloproteinase-9	P14780	5.1 (4.6 -- 5.7)	5.3 (4.8 -- 5.8)	1.3
MPO	Myeloperoxidase	P05164	3.0 (2.8 -- 3.3)	3.0 (2.8 -- 3.3)	0.6
Notch 3	Neurogenic locus notch homolog protein 3	Q9UM47	5.7 (5.4 -- 6.0)	5.8 (5.5 -- 6.0)	1.3
NT-proBNP	N-terminal prohormone brain natriuretic peptide	NA	6.7 (5.9 -- 7.3)	6.9 (6.1 -- 7.7)	2.2
OPG	Osteoprotegerin	O00300	3.9 (3.7 -- 4.2)	4.0 (3.8 -- 4.3)	1.5
OPN	Osteopontin	P10451	7.4 (7.0 -- 7.8)	7.6 (7.2 -- 8.0)	1.3
PAI	Plasminogen activator inhibitor 1	P05121	5.3 (4.7 -- 6.0)	5.4 (4.7 -- 6.1)	1.0
PCSK9	Proprotein convertase subtilisin/kexin type 9	Q8NBP7	3.1 (2.9 -- 3.4)	3.2 (2.9 -- 3.4)	0.9
PDGF subunit A	Platelet-derived growth factor subunit A	P04085	3.1 (2.6 -- 3.8)	3.4 (2.7 -- 4.1)	1.3
PECAM-1	Platelet endothelial cell adhesion molecule	P16284	4.1 (3.9 -- 4.3)	4.2 (4.0 -- 4.4)	1.0
PGLYRP1	Peptidoglycan recognition protein 1	O75594	7.3 (6.9 -- 7.6)	7.4 (7.0 -- 7.7)	0.4
PI3	Elafin	P19957	2.4 (2.0 -- 2.9)	2.5 (2.1 -- 3.1)	-0.0
PLC	Perlecan	P98160	7.9 (7.7 -- 8.1)	7.9 (7.7 -- 8.2)	1.0
PON3	Paraoxonase	Q15166	5.7 (5.3 -- 6.1)	5.7 (5.4 -- 6.1)	1.0
PRTN3	Myeloblastin	P24158	3.7 (3.4 -- 4.1)	3.8 (3.4 -- 4.1)	0.3
PSP-D	Pulmonary surfactant-associated protein D	P35247	3.2 (2.6 -- 3.8)	3.3 (2.8 -- 3.8)	1.2
RARRES2	Retinoic acid receptor responder protein 2	Q99969	11.4 (11.3 -- 11.6)	11.5 (11.3 -- 11.6)	1.9
RETN	Resistin	Q9HD89	6.0 (5.7 -- 6.3)	6.1 (5.8 -- 6.4)	0.6
SCGB3A2	Secretoglobin family 3A member 2	Q96PL1	2.7 (2.3 -- 3.2)	2.7 (2.3 -- 3.2)	0.9
SELE	E-selectin	P16581	11.7 (11.3 -- 12.1)	11.8 (11.4 -- 12.2)	3.5
SELP	P-selectin	P16109	9.3 (9.0 -- 9.7)	9.4 (9.2 -- 9.8)	1.7

SHPS-1	Tyrosine-protein phosphatase non- receptor type substrate 1	P78324	3.6 (3.3 -- 3.9)	3.7 (3.4 -- 4.0)	1.3
SPON1	Spondin-1	Q9HCB6	2.3 (2.1 -- 2.5)	2.4 (2.2 -- 2.6)	1.6
ST2	ST2 protein	Q01638	4.5 (4.1 -- 4.8)	4.5 (4.2 -- 4.9)	1.4
t-PA	Tissue-type plasminogen activator	P00750	6.8 (6.4 -- 7.1)	6.8 (6.3 -- 7.2)	1.7
TFF3	Trefoil factor 3	Q07654	5.1 (4.8 -- 5.4)	5.2 (5.0 -- 5.5)	1.3
TFPI	Tissue factor pathway inhibitor	P10646	8.9 (8.7 -- 9.2)	9.0 (8.8 -- 9.2)	1.2
TIMP4	Metalloproteinase inhibitor 4	Q99727	3.7 (3.4 -- 4.0)	3.7 (3.4 -- 4.0)	1.2
TLT-2	Trem-like transcript 2 protein	Q5T2D2	4.7 (4.4 -- 5.0) [2]	4.7 (4.5 -- 5.0) [0]	2.1
TNF-R1	Tumor necrosis factor receptor 1	P19438	6.7 (6.4 -- 7.0)	6.9 (6.5 -- 7.2)	1.6
TNF-R2	Tumor necrosis factor receptor 2	P20333	5.7 (5.4 -- 6.0)	5.8 (5.5 -- 6.2)	1.8
TNFRSF10C	Tumor necrosis factor receptor superfamily member 10C	O14798	6.7 (6.4 -- 7.1)	6.8 (6.3 -- 7.1)	1.8
TNFRSF14	Tumor necrosis factor receptor superfamily member 14	Q92956	4.5 (4.3 -- 4.8)	4.7 (4.3 -- 4.9)	1.4
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B	Q9Y275	6.9 (6.7 -- 7.2)	7.0 (6.8 -- 7.2)	1.4
TR	Transferrin receptor protein 1	P02786	5.6 (5.1 -- 6.0)	5.6 (5.0 -- 6.1)	0.8
TR-AP	Tartrate-resistant acid phosphatase type 5	P13686	3.1 (2.9 -- 3.4)	3.1 (2.8 -- 3.3)	-0.4
U-PAR	Urokinase plasminogen activator surface receptor	Q03405	5.5 (5.2 -- 5.7)	5.6 (5.4 -- 5.9)	1.1
uPA	Urokinase-type plasminogen activator	P00749	4.6 (4.4 -- 4.8)	4.6 (4.4 -- 4.9)	0.1
vWF	von Willebrand factor	P04275	6.2 (5.7 -- 6.7)	6.2 (5.7 -- 6.7)	1.8

Continuous variables presented as median (Q1-Q3). Number of missing values presented in [n]. SE, systemic embolism.
The CVDII panel were used for biomarkers ranging from ACE2 to XCL1; CVDIII panel for ALCAM to vWF.

Table S3. Spearman correlation between selected proximity extension assay (PEA) biomarkers and established biomarkers in identification cohort.

	Cystatin C	NT-proBNP	Troponin T
CCL16	0,326057	0,116803	0,216475
TFPI	0,116171	0,027273	0,071213
CTSZ	0,419811	0,089237	0,255986
vWF	0,223911	0,128312	0,156687
OPN	0,369751	0,279303	0,339888
LDL receptor	-0,01631	-0,23105	-0,14058
RARRES2	0,384146	0,065364	0,167948
PAI	-0,01812	-0,10743	-0,05619
TGM2	0,130266	0,034768	0,054574
BLM hydrolase	0,012694	0,026024	0,046392
IL-8	0,229656	0,158906	0,210904
t-PA	0,142776	0,00769	0,070268
ST2	0,129105	0,233633	0,227892
CTSL1	0,195729	0,13611	0,18377
ICAM-2	0,218761	0,103348	0,167416
THPO	0,104614	-0,02697	0,019508
RETN	0,423148	0,184607	0,243007
EGFR	-0,17501	-0,22745	-0,18511
SORT1	0,102739	0,16729	0,128806
PECAM-1	0,139443	0,053568	0,108929
CD163	0,209344	0,04723	0,109585
MCP-3	0,251239	0,067235	0,197417
PI3	0,43205	0,184406	0,330276
CASP-8	0,174668	0,061145	0,099539
AXL	0,325201	0,149479	0,188641
MMP-9	0,1419	0,028383	0,05646
SPON1	0,36497	0,37444	0,34543
PARP-1	0,163689	0,067256	0,083399
SLAMF7	0,193701	0,18533	0,162963
ADAM-TS13	-0,13264	-0,1052	-0,13527
GIF	0,081056	-0,03373	0,059539
IL-17D	0,256782	0,280115	0,227042
Protein BOC	0,03653	0,129242	0,12147
REN	0,20946	-0,06636	0,161811

Correlation analyses for the PEA biomarkers associated with ischemic stroke according to adjusted Cox-regression analyses (model B). Cystatin C represents renal function, N-terminal prohormone brain natriuretic peptide (NT-proBNP) cardiac dysfunction, and troponin T myocyte damage.

Table S4. Spearman correlation between selected proximity extension assay (PEA) biomarkers and established biomarkers in validation cohort.

	Cystatin C	NT-proBNP	Troponin T
TIM	0,199422	0,137887	0,222042
MMP-12	0,263789	0,210639	0,218399
Ig G Fc receptor II-b	0,108278	0,094566	0,046282
Gal-9	0,542171	0,221024	0,194315
SHPS-1	0,236335	0,102631	0,115044
MMP-9	0,128594	0,021436	0,059973
IL-1RT1	0,140452	0,205891	0,229196
PDGF subunit B	0,033149	0,012021	-0,03668
ALCAM	0,217539	0,153672	0,07557
PD-L2	0,22183	0,176829	0,164531
PRSS27	0,173877	-0,01033	0,057556
IL-1ra	0,275154	0,028785	0,032681
GDF-15	0,461178	0,358954	0,472604
PDGF subunit A	0,039029	0,02956	-0,02062
CD40-L	0,078597	0,039913	-0,01413
Dkk-1	0,125303	0,049636	0,038688
SORT1	0,037477	0,1309	0,08599
IL-18	0,18602	0,106796	0,091669
GT	0,228407	0,176812	0,120412

Correlation analyses for the PEA biomarkers associated with ischemic stroke according to adjusted Cox-regression analyses (model B). Cystatin C represents renal function, N-terminal prohormone brain natriuretic peptide (NT-proBNP) cardiac dysfunction, and troponin T myocyte damage.

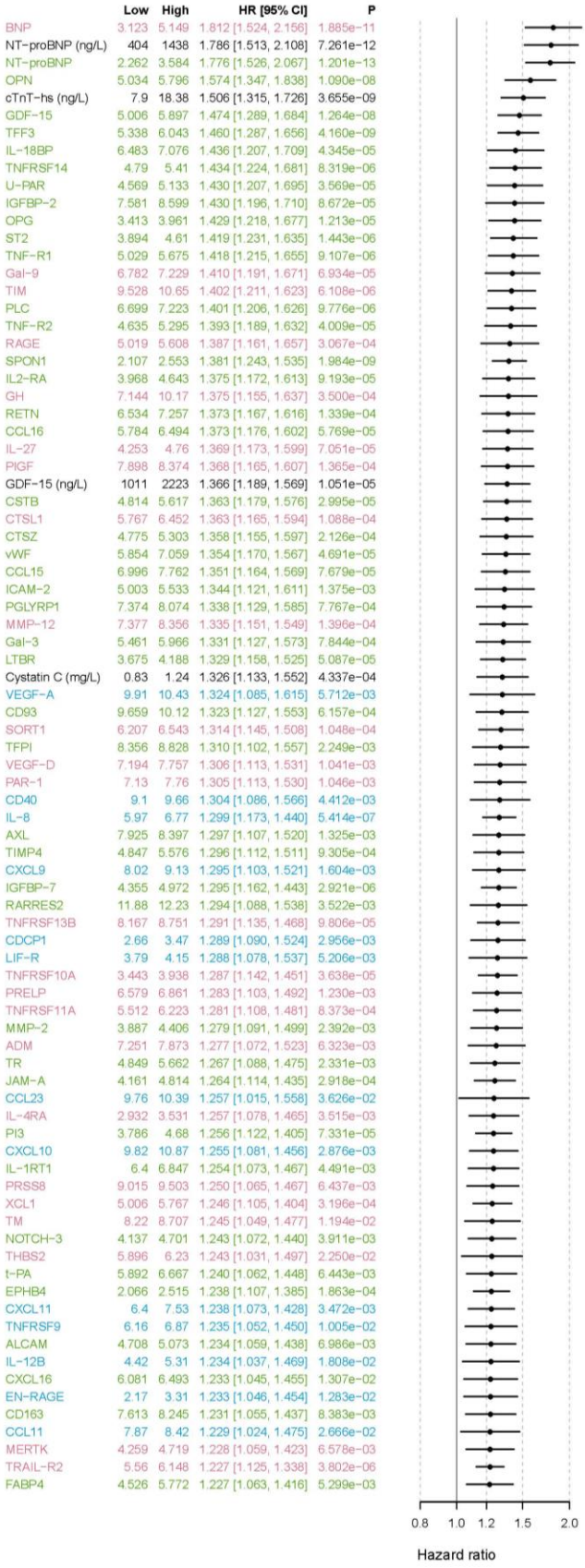
Table S5. Baseline concentrations of the identified proximity extension assay (PEA) biomarkers associated with ischemic stroke/systemic embolism (SE).

Identification cohort	**No event (n=4,124)**	**Ischemic stroke/SE (n=282)**	***p*-value**
MMP9	4.2 (3.7 -- 4.7) [23]	4.3 (3.7 -- 4.7) [0]	0.0592
OPN	5.4 (5.0 -- 5.7) [23]	5.6 (5.2 -- 5.9) [0]	1.09E-08
SORT1	6.4 (6.2 -- 6.5) [203]	6.4 (6.2 -- 6.6) [12]	1.05E-04
ST2	4.2 (3.9 -- 4.6) [23]	4.4 (4.0 -- 4.7) [0]	1.44E-06
TFF3	5.6 (5.3 -- 6.0) [23]	5.7 (5.4 -- 6.2) [0]	4.16E-09
Validation cohort	**No event (n=1,062)**	**Ischemic stroke/SE (n=149)**	
MMP9	5.1 (4.6 -- 5.7)	5.3 (4.8 -- 5.8)	0.0155
OPN	7.4 (7.0 -- 7.8)	7.6 (7.2 -- 8.0)	0.0028
SORT1	8.5 (8.3 -- 8.7)	8.6 (8.4 -- 8.8)	0.0041
ST2	4.5 (4.1 -- 4.8)	4.5 (4.2 -- 4.9)	0.1278
TFF3	5.1 (4.8 -- 5.4)	5.2 (5.0 -- 5.5)	4.95E-04

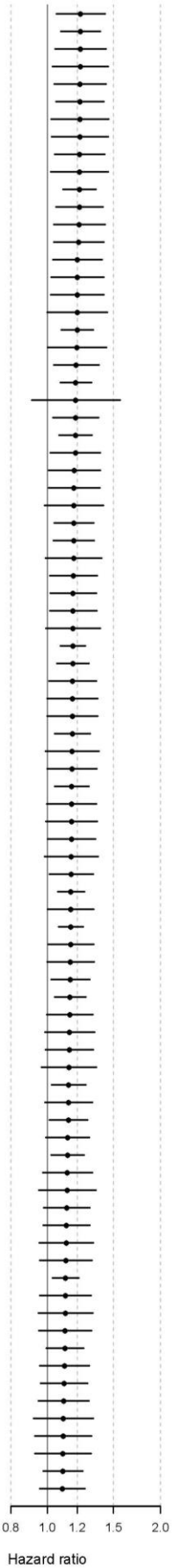
P-value according to unadjusted Cox-regression models. Number of missing values presented in [n]. MMP9, matrix metalloproteinase-9; OPN, osteopontin; SORT1, sortilin; ST2, suppression of tumorigenesis 2; TFF3, trefoil factor-3.

Figure S1A

Association of all 255 biomarkers with ischemic stroke or systemic embolism according to unadjusted Cox-regression analysis in the identification cohort



	Low	High	HR [95% CI]	P
PECAM−1	4.542	5.01	1.226 [1.055, 1.426]	0.0079242
DCN	5.277	5.634	1.226 [1.084, 1.387]	0.0011916
CTSD	4.024	4.688	1.226 [1.048, 1.434]	0.0110818
TGM2	7.805	8.776	1.224 [1.029, 1.456]	0.0222404
FGF−21	6.872	8.755	1.222 [1.042, 1.433]	0.0136773
Dkk−1	8.509	9.333	1.222 [1.053, 1.418]	0.0083487
CHI3L1	6.818	8.237	1.221 [1.023, 1.457]	0.0271143
SHPS−1	3.682	4.303	1.221 [1.025, 1.454]	0.0253207
BLM hydrolase	5.505	6.006	1.219 [1.044, 1.424]	0.0120848
CD5	3.2	3.72	1.219 [1.021, 1.456]	0.0287880
MCP−3	1.92	2.46	1.219 [1.100, 1.350]	0.0001573
GRN	3.321	3.711	1.217 [1.053, 1.408]	0.0078612
IL−15RA	0.92	1.29	1.217 [1.039, 1.426]	0.0149617
Gal−4	3.455	4.181	1.212 [1.039, 1.415]	0.0144783
ACE2	3.58	4.435	1.203 [1.033, 1.401]	0.0173373
BMP−6	5.438	6.044	1.203 [1.022, 1.415]	0.0263728
MMP−3	7.05	7.949	1.201 [1.018, 1.418]	0.0295848
CX3CL1	5.49	6.09	1.201 [0.998, 1.445]	0.0519215
HGF	7.27	7.85	1.201 [1.086, 1.328]	0.0003413
IGFBP−1	4.48	6.325	1.200 [1.000, 1.440]	0.0495881
TGF−alpha	0.98	1.44	1.194 [1.038, 1.373]	0.0130365
Beta−NGF	1.44	1.78	1.191 [1.080, 1.312]	0.0004361
CSF−1	7.84	8.15	1.191 [0.907, 1.563]	0.2090597
CASP−8	2.36	3.53	1.190 [1.034, 1.370]	0.0154585
FGF−5	1.74	2.08	1.189 [1.074, 1.317]	0.0008830
CNTN1	2.989	3.392	1.188 [1.018, 1.387]	0.0290658
hOSCAR	9.608	9.918	1.180 [1.005, 1.386]	0.0428695
TF	5.585	6.017	1.178 [1.005, 1.381]	0.0433372
Ig G Fc receptor II−b	1.376	2.095	1.178 [0.983, 1.411]	0.0768514
AGRP	3.009	3.548	1.177 [1.042, 1.329]	0.0088091
CDH5	3.529	4.034	1.176 [1.037, 1.334]	0.0117633
IL−18	8.367	9.073	1.176 [0.988, 1.398]	0.0677998
LOX−1	6.553	7.33	1.173 [1.013, 1.358]	0.0332422
IL−6 (ng/L)	1.6	4.3	1.172 [1.016, 1.352]	0.0293051
CEACAM8	3.886	4.714	1.171 [1.013, 1.354]	0.0325967
MMP−7	6.297	8.193	1.171 [0.990, 1.386]	0.0655787
SLAMF7	2.387	2.793	1.170 [1.081, 1.265]	0.0000979
CCL28	0.85	1.26	1.169 [1.059, 1.291]	0.0019728
THPO	1.932	2.35	1.167 [1.007, 1.353]	0.0399564
LAP TGF−beta−1	6.1	6.68	1.167 [0.997, 1.364]	0.0540151
COL1A1	2.205	2.774	1.166 [0.997, 1.363]	0.0543152
FGF−23	3.872	4.887	1.166 [1.044, 1.302]	0.0063325
PDGF subunit A	2.443	3.745	1.164 [0.987, 1.374]	0.0716811
VSIG2	3.656	4.385	1.164 [0.999, 1.356]	0.0519501
CD4	4.506	4.957	1.161 [1.044, 1.291]	0.0059833
MMP−9	3.71	4.736	1.160 [0.994, 1.354]	0.0591805
TLT−2	3.9	4.545	1.160 [0.989, 1.359]	0.0674526
4E−BP1	7.87	9.17	1.160 [0.999, 1.346]	0.0512082
PRSS27	7.825	8.427	1.159 [0.982, 1.368]	0.0808487
PAPPA	2.918	3.6	1.158 [1.010, 1.328]	0.0348927
FAS	4.815	5.265	1.156 [1.063, 1.258]	0.0007166
PRTN3	4.426	5.142	1.155 [1.002, 1.332]	0.0473869
CCL3	2.548	3.133	1.155 [1.070, 1.246]	0.0002134
CD84	5.101	5.646	1.155 [1.003, 1.329]	0.0451959
IL−1ra	4.139	5.069	1.153 [0.997, 1.333]	0.0548135
SCGB3A2	2.179	3.224	1.152 [1.022, 1.299]	0.0201051
PD−L2	2.898	3.373	1.150 [1.045, 1.266]	0.0042930
SPON2	8.887	9.134	1.148 [0.995, 1.325]	0.0580774
MCP−4	1.95	2.68	1.147 [0.983, 1.338]	0.0808839
TNFSF14	2.31	3	1.145 [0.987, 1.327]	0.0736590
PTX3	3.404	4.033	1.142 [0.963, 1.353]	0.1261956
PARP−1	3.021	3.819	1.141 [1.026, 1.269]	0.0151762
PIgR	6.367	6.551	1.140 [0.984, 1.320]	0.0803206
HB−EGF	5.612	6.08	1.138 [1.012, 1.281]	0.0314802
AP−N	4.949	5.362	1.133 [0.990, 1.296]	0.0687019
NT−3	1.68	2.23	1.132 [1.022, 1.253]	0.0169207
MEPE	3.069	3.699	1.131 [0.970, 1.319]	0.1159226
MCP−2	8.61	9.42	1.131 [0.948, 1.350]	0.1728521
SELP	8.746	9.517	1.126 [0.977, 1.298]	0.1012563
hGDNF	2.04	2.52	1.124 [0.974, 1.297]	0.1088868
TNFSF13B	6.274	6.799	1.123 [0.952, 1.325]	0.1699849
FS	11.71	12.3	1.121 [0.953, 1.318]	0.1680130
MCP−1	3.168	3.631	1.119 [1.030, 1.215]	0.0075650
CASP−3	5.724	7.767	1.118 [0.953, 1.311]	0.1703272
PAI	5.591	6.889	1.118 [0.944, 1.323]	0.1953609
CCL19	8.99	10.06	1.115 [0.947, 1.313]	0.1917928
CCL4	5.51	6.19	1.115 [0.993, 1.251]	0.0652973
SLAMF1	3.19	3.89	1.111 [0.953, 1.296]	0.1788566
SIRT2	3.44	5.36	1.108 [0.958, 1.283]	0.1671617
HO−1	11.38	11.92	1.105 [0.946, 1.291]	0.2062124
IL−18R1	7.25	7.82	1.103 [0.918, 1.326]	0.2943986
ANG−1	8.109	9.612	1.103 [0.925, 1.314]	0.2742374
CXCL5	9.432	11.73	1.101 [0.925, 1.310]	0.2779133
ITGB1BP2	3.021	4.515	1.100 [0.974, 1.243]	0.1235610
CA5A	2.076	3.278	1.096 [0.954, 1.261]	0.1960897



	Low	High	HR [95% CI]	P
STK4	1.168	3.496	1.094 [0.898, 1.333]	3.736e−01
AZU1	2.569	3.695	1.093 [0.955, 1.251]	1.960e−01
CCL20	6.09	7.41	1.092 [0.963, 1.239]	1.711e−01
CD40−L	3.543	5.126	1.092 [0.934, 1.276]	2.694e−01
IL−6RA	10.7	11.27	1.091 [0.932, 1.278]	2.791e−01
STAMPB	2.93	4.107	1.091 [0.961, 1.239]	1.797e−01
IL−10RB	6.5	6.97	1.090 [0.870, 1.365]	4.560e−01
LDL receptor	4.17	5.02	1.089 [0.932, 1.273]	2.820e−01
MPO	4.119	4.697	1.089 [0.954, 1.243]	2.063e−01
MMP−10	8.9	9.7	1.088 [0.913, 1.297]	3.467e−01
IL−17RA	3.841	4.475	1.086 [0.937, 1.259]	2.733e−01
CCL17	6.251	7.63	1.086 [0.923, 1.278]	3.215e−01
IL1RL2	4.322	4.899	1.085 [0.926, 1.271]	3.154e−01
PD−L1	4.77	5.33	1.083 [0.950, 1.235]	2.325e−01
ST1A1	0.5	2.667	1.082 [0.893, 1.311]	4.217e−01
OSM	2.14	3.17	1.082 [0.930, 1.258]	3.075e−01
CD6	3.66	4.25	1.081 [0.906, 1.290]	3.866e−01
IL16	5.043	5.659	1.080 [0.928, 1.257]	3.204e−01
AMBP	6.976	7.249	1.080 [0.904, 1.290]	3.979e−01
IL−6	3.532	4.642	1.080 [0.955, 1.221]	2.216e−01
CPB1	3.708	4.627	1.079 [0.915, 1.274]	3.657e−01
AXIN1	1.36	2.54	1.079 [0.948, 1.228]	2.472e−01
PDGF subunit B	8.058	9.551	1.078 [0.909, 1.279]	3.864e−01
CST5	6.65	7.42	1.078 [0.889, 1.308]	4.439e−01
ITGB2	5.449	6.001	1.077 [0.933, 1.243]	3.114e−01
IL−7	2.743	3.8	1.076 [0.913, 1.269]	3.827e−01
MB	6.883	7.684	1.073 [0.925, 1.245]	3.538e−01
MMP−1	6.82	8.33	1.072 [0.918, 1.253]	3.779e−01
TIE2	7.931	8.265	1.072 [0.923, 1.245]	3.602e−01
CXCL6	6.802	7.93	1.071 [0.914, 1.253]	3.972e−01
IL−17D	2.573	2.956	1.068 [0.982, 1.161]	1.250e−01
CHIT1	2.576	4.202	1.066 [0.925, 1.228]	3.770e−01
SERPINA12	3.043	4.379	1.064 [0.935, 1.210]	3.462e−01
PSP−D	2.286	3.315	1.063 [0.898, 1.258]	4.798e−01
NEMO	3.735	5.073	1.062 [0.916, 1.230]	4.270e−01
DECR1	2.536	3.858	1.060 [0.906, 1.241]	4.657e−01
GT	1.583	2.367	1.058 [0.926, 1.209]	4.092e−01
LPL	9.244	9.795	1.056 [0.917, 1.216]	4.463e−01
CXCL1	7.429	8.906	1.055 [0.883, 1.261]	5.553e−01
CPA1	4.166	5.107	1.046 [0.895, 1.224]	5.705e−01
DLK−1	4.952	5.833	1.045 [0.890, 1.228]	5.918e−01
uPA	4.803	5.242	1.045 [0.940, 1.162]	4.152e−01
FGF−19	7.39	8.848	1.038 [0.870, 1.240]	6.769e−01
HSP 27	9.608	10.61	1.038 [0.871, 1.236]	6.776e−01
MARCO	6.059	6.378	1.034 [0.914, 1.169]	5.990e−01
CD244	5.79	6.22	1.032 [0.867, 1.228]	7.243e−01
TR−AP	4.686	5.259	1.032 [0.879, 1.211]	7.038e−01
GLO1	5.709	6.688	1.031 [0.885, 1.202]	6.935e−01
KLK6	3.547	3.64	1.031 [0.993, 1.070]	1.092e−01
IL−17A	0.45	0.81	1.025 [0.952, 1.104]	5.055e−01
GDF−2	4.233	4.941	1.025 [0.887, 1.184]	7.350e−01
SOD2	8.692	9.019	1.023 [0.859, 1.218]	7.985e−01
CCL24	5.218	6.455	1.023 [0.861, 1.215]	7.968e−01
SELE	2.11	2.846	1.022 [0.871, 1.201]	7.862e−01
CCL25	6.54	7.33	1.022 [0.855, 1.220]	8.127e−01
HAOX1	3.713	5.71	1.018 [0.857, 1.210]	8.392e−01
ADA	3.88	4.4	1.008 [0.850, 1.194]	9.288e−01
IL−10	4.03	4.637	1.006 [0.915, 1.107]	9.024e−01
Ep−CAM	3.482	4.642	1.004 [0.864, 1.166]	9.602e−01
EGFR	1.759	2.047	1.003 [0.879, 1.144]	9.677e−01
SRC	4.382	6.943	1.002 [0.807, 1.243]	9.877e−01
IL−17C	1.54	1.86	1.000 [0.925, 1.082]	9.921e−01
TNFRSF10C	5.759	6.41	0.998 [0.868, 1.148]	9.807e−01
TNFB	2.81	3.32	0.997 [0.853, 1.164]	9.662e−01
IL−1RT2	4.999	5.459	0.992 [0.878, 1.120]	8.953e−01
FABP2	8.602	9.743	0.988 [0.837, 1.166]	8.864e−01
CCL22	1.974	2.768	0.987 [0.894, 1.089]	7.891e−01
SCF	9.35	9.901	0.985 [0.826, 1.175]	8.640e−01
IL−10RA	0.86	0.92	0.985 [0.963, 1.007]	1.802e−01
Flt3L	9.06	9.61	0.977 [0.827, 1.153]	7.810e−01
PSGL−1	4.802	5.111	0.975 [0.866, 1.098]	6.770e−01
PCSK9	2.649	3.193	0.972 [0.846, 1.116]	6.865e−01
TWEAK	8.06	8.48	0.947 [0.812, 1.103]	4.826e−01
LEP	5.954	7.377	0.938 [0.804, 1.093]	4.096e−01
Protein BOC	4.686	5.043	0.918 [0.800, 1.054]	2.233e−01
TRAIL	7.43	7.82	0.913 [0.774, 1.075]	2.747e−01
CTRC	9.707	10.69	0.910 [0.785, 1.056]	2.162e−01
DNER	6.89	7.3	0.904 [0.758, 1.078]	2.618e−01
TRANCE	4.14	5.03	0.902 [0.754, 1.080]	2.615e−01
IDUA	4.102	4.953	0.895 [0.768, 1.042]	1.520e−01
BDNF	2.38	6.03	0.881 [0.738, 1.052]	1.612e−01
PON3	5.244	6.056	0.871 [0.754, 1.007]	6.240e−02
ADAM−TS13	5.078	5.268	0.845 [0.780, 0.915]	3.291e−05
GIF	4.936	6.204	0.840 [0.723, 0.977]	2.325e−02
REN	6.796	8.21	0.820 [0.690, 0.974]	2.353e−02

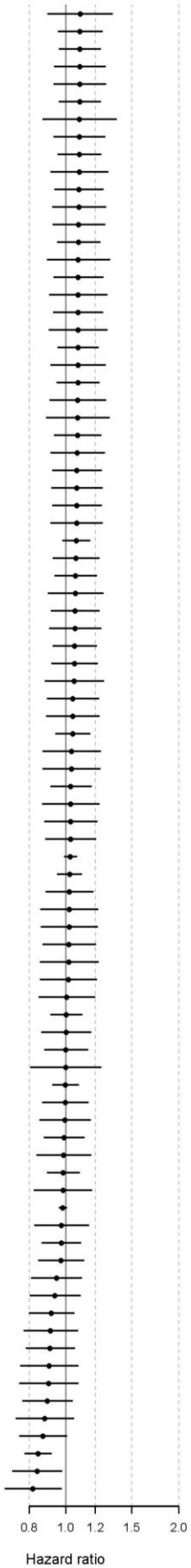
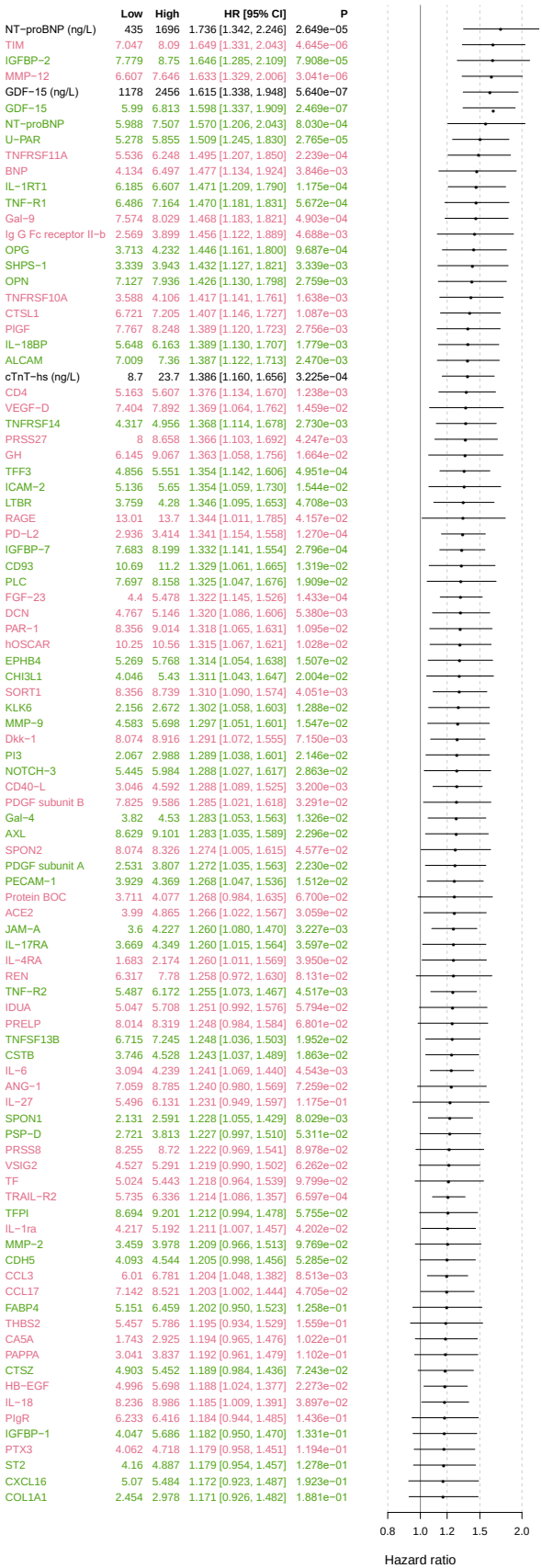


Figure S1B

Association of all 188 biomarkers with ischemic stroke or systemic embolism according to unadjusted Cox-regression analysis in the validation cohort



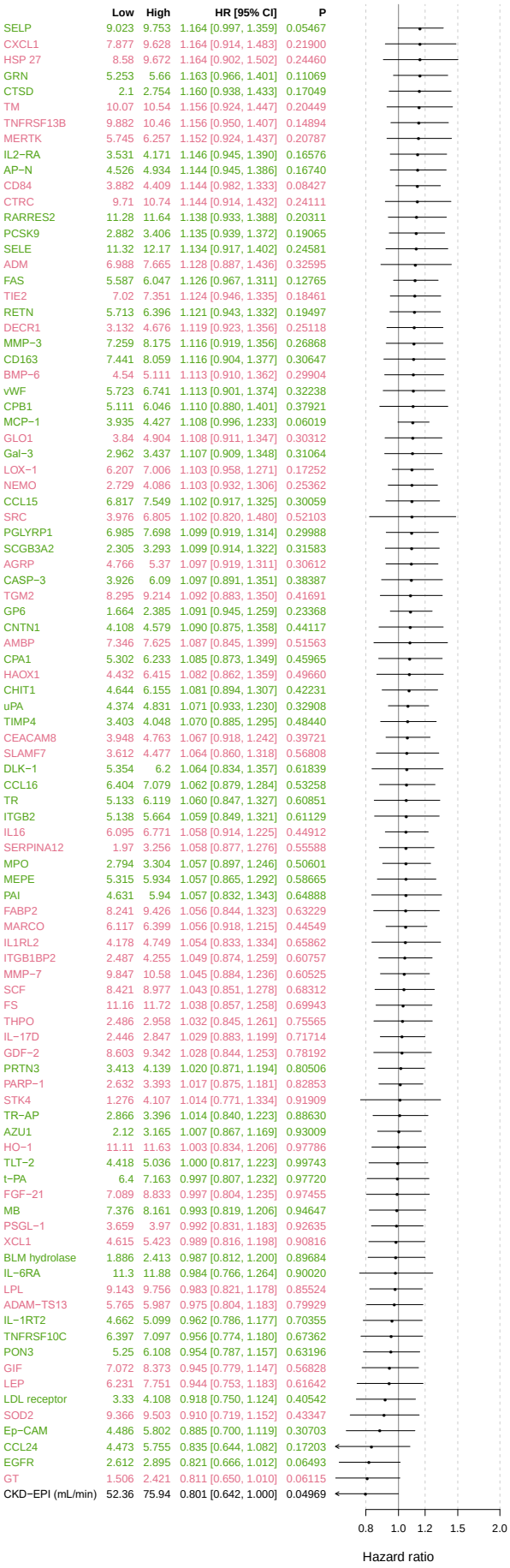

Low High HR [95% CI] P
SELP 9.023 9.753 1.164 [0.997, 1.359] 0.05467
CXCL1 7.877 9.628 1.164 [0.914, 1.483] 0.21900
HSP 27 8.58 9.672 1.164 [0.902, 1.502] 0.24460
GRN 5.253 5.66 1.163 [0.966, 1.401] 0.11069
CTSD 2.1 2.754 1.160 [0.938, 1.433] 0.17049
TM 10.07 10.54 1.156 [0.924, 1.447] 0.20449
TNFRSF13B 9.882 10.46 1.156 [0.950, 1.407] 0.14894
MERTK 5.745 6.257 1.152 [0.924, 1.437] 0.20787
IL2−RA 3.531 4.171 1.146 [0.945, 1.390] 0.16576
AP−N 4.526 4.934 1.144 [0.945, 1.386] 0.16740
CD84 3.882 4.409 1.144 [0.982, 1.333] 0.08427
CTRC 9.71 10.74 1.144 [0.914, 1.432] 0.24111
RARRES2 11.28 11.64 1.138 [0.933, 1.388] 0.20311
PCSK9 2.882 3.406 1.135 [0.939, 1.372] 0.19065
SELE 11.32 12.17 1.134 [0.917, 1.402] 0.24581
ADM 6.988 7.665 1.128 [0.887, 1.436] 0.32595
FAS 5.587 6.047 1.126 [0.967, 1.311] 0.12765
TIE2 7.02 7.351 1.124 [0.946, 1.335] 0.18461
RETN 5.713 6.396 1.121 [0.943, 1.332] 0.19497
DECR1 3.132 4.676 1.119 [0.923, 1.356] 0.25118
MMP−3 7.259 8.175 1.116 [0.919, 1.356] 0.26868
CD163 7.441 8.059 1.116 [0.904, 1.377] 0.30647
BMP−6 4.54 5.111 1.113 [0.910, 1.362] 0.29904
vWF 5.723 6.741 1.113 [0.901, 1.374] 0.32238
CPB1 5.111 6.046 1.110 [0.880, 1.401] 0.37921
MCP−1 3.935 4.427 1.108 [0.996, 1.233] 0.06019
GLO1 3.84 4.904 1.108 [0.911, 1.347] 0.30312
Gal−3 2.962 3.437 1.107 [0.909, 1.348] 0.31064
LOX−1 6.207 7.006 1.103 [0.958, 1.271] 0.17252
NEMO 2.729 4.086 1.103 [0.932, 1.306] 0.25362
CCL15 6.817 7.549 1.102 [0.917, 1.325] 0.30059
SRC 3.976 6.805 1.102 [0.820, 1.480] 0.52103
PGLYRP1 6.985 7.698 1.099 [0.919, 1.314] 0.29988
SCGB3A2 2.305 3.293 1.099 [0.914, 1.322] 0.31583
AGRP 4.766 5.37 1.097 [0.919, 1.311] 0.30612
CASP−3 3.926 6.09 1.097 [0.891, 1.351] 0.38387
TGM2 8.295 9.214 1.092 [0.883, 1.350] 0.41691
GP6 1.664 2.385 1.091 [0.945, 1.259] 0.23368
CNTN1 4.108 4.579 1.090 [0.875, 1.358] 0.44117
AMBP 7.346 7.625 1.087 [0.845, 1.399] 0.51563
CPA1 5.302 6.233 1.085 [0.873, 1.349] 0.45965
HAOX1 4.432 6.415 1.082 [0.862, 1.359] 0.49660
CHIT1 4.644 6.155 1.081 [0.894, 1.307] 0.42231
uPA 4.374 4.831 1.071 [0.933, 1.230] 0.32908
TIMP4 3.403 4.048 1.070 [0.885, 1.295] 0.48440
CEACAM8 3.948 4.763 1.067 [0.918, 1.242] 0.39721
SLAMF7 3.612 4.477 1.064 [0.860, 1.318] 0.56808
DLK−1 5.354 6.2 1.064 [0.834, 1.357] 0.61839
CCL16 6.404 7.079 1.062 [0.879, 1.284] 0.53258
TR 5.133 6.119 1.060 [0.847, 1.327] 0.60851
ITGB2 5.138 5.664 1.059 [0.849, 1.321] 0.61129
IL16 6.095 6.771 1.058 [0.914, 1.225] 0.44912
SERPINA12 1.97 3.256 1.058 [0.877, 1.276] 0.55588
MPO 2.794 3.304 1.057 [0.897, 1.246] 0.50601
MEPE 5.315 5.934 1.057 [0.865, 1.292] 0.58665
PAI 4.631 5.94 1.057 [0.832, 1.343] 0.64888
FABP2 8.241 9.426 1.056 [0.844, 1.323] 0.63229
MARCO 6.117 6.399 1.056 [0.918, 1.215] 0.44549
IL1RL2 4.178 4.749 1.054 [0.833, 1.334] 0.65862
ITGB1BP2 2.487 4.255 1.049 [0.874, 1.259] 0.60757
MMP−7 9.847 10.58 1.045 [0.884, 1.236] 0.60525
SCF 8.421 8.977 1.043 [0.851, 1.278] 0.68312
FS 11.16 11.72 1.038 [0.857, 1.258] 0.69943
THPO 2.486 2.958 1.032 [0.845, 1.261] 0.75565
IL−17D 2.446 2.847 1.029 [0.883, 1.199] 0.71714
GDF−2 8.603 9.342 1.028 [0.844, 1.253] 0.78192
PRTN3 3.413 4.139 1.020 [0.871, 1.194] 0.80506
PARP−1 2.632 3.393 1.017 [0.875, 1.181] 0.82853
STK4 1.276 4.107 1.014 [0.771, 1.334] 0.91909
TR−AP 2.866 3.396 1.014 [0.840, 1.223] 0.88630
AZU1 2.12 3.165 1.007 [0.867, 1.169] 0.93009
HO−1 11.11 11.63 1.003 [0.834, 1.206] 0.97786
TLT−2 4.418 5.036 1.000 [0.817, 1.223] 0.99743
t−PA 6.4 7.163 0.997 [0.807, 1.232] 0.97720
FGF−21 7.089 8.833 0.997 [0.804, 1.235] 0.97455
MB 7.376 8.161 0.993 [0.819, 1.206] 0.94647
PSGL−1 3.659 3.97 0.992 [0.831, 1.183] 0.92635
XCL1 4.615 5.423 0.989 [0.816, 1.198] 0.90816
BLM hydrolase 1.886 2.413 0.987 [0.812, 1.200] 0.89684
IL−6RA 11.3 11.88 0.984 [0.766, 1.264] 0.90020
LPL 9.143 9.756 0.983 [0.821, 1.178] 0.85524
ADAM−TS13 5.765 5.987 0.975 [0.804, 1.183] 0.79929
IL−1RT2 4.662 5.099 0.962 [0.786, 1.177] 0.70355
TNFRSF10C 6.397 7.097 0.956 [0.774, 1.180] 0.67362
PON3 5.25 6.108 0.954 [0.787, 1.157] 0.63196
GIF 7.072 8.373 0.945 [0.779, 1.147] 0.56828
LEP 6.231 7.751 0.944 [0.753, 1.183] 0.61642
LDL receptor 3.33 4.108 0.918 [0.750, 1.124] 0.40542
SOD2 9.366 9.503 0.910 [0.719, 1.152] 0.43347
Ep−CAM 4.486 5.802 0.885 [0.700, 1.119] 0.30703
CCL24 4.473 5.755 0.835 [0.644, 1.082] 0.17203
EGFR 2.612 2.895 0.821 [0.666, 1.012] 0.06493
GT 1.506 2.421 0.811 [0.650, 1.010] 0.06115
CKD−EPI (mL/min) 52.36 75.94 0.801 [0.642, 1.000] 0.04969
0.8 1.0 1.2 1.5 2.0
Hazard ratio

Figure S2A

Association of the top biomarkers (Table 3) with ischemic stroke/systemic embolism by using splines in the identification cohort

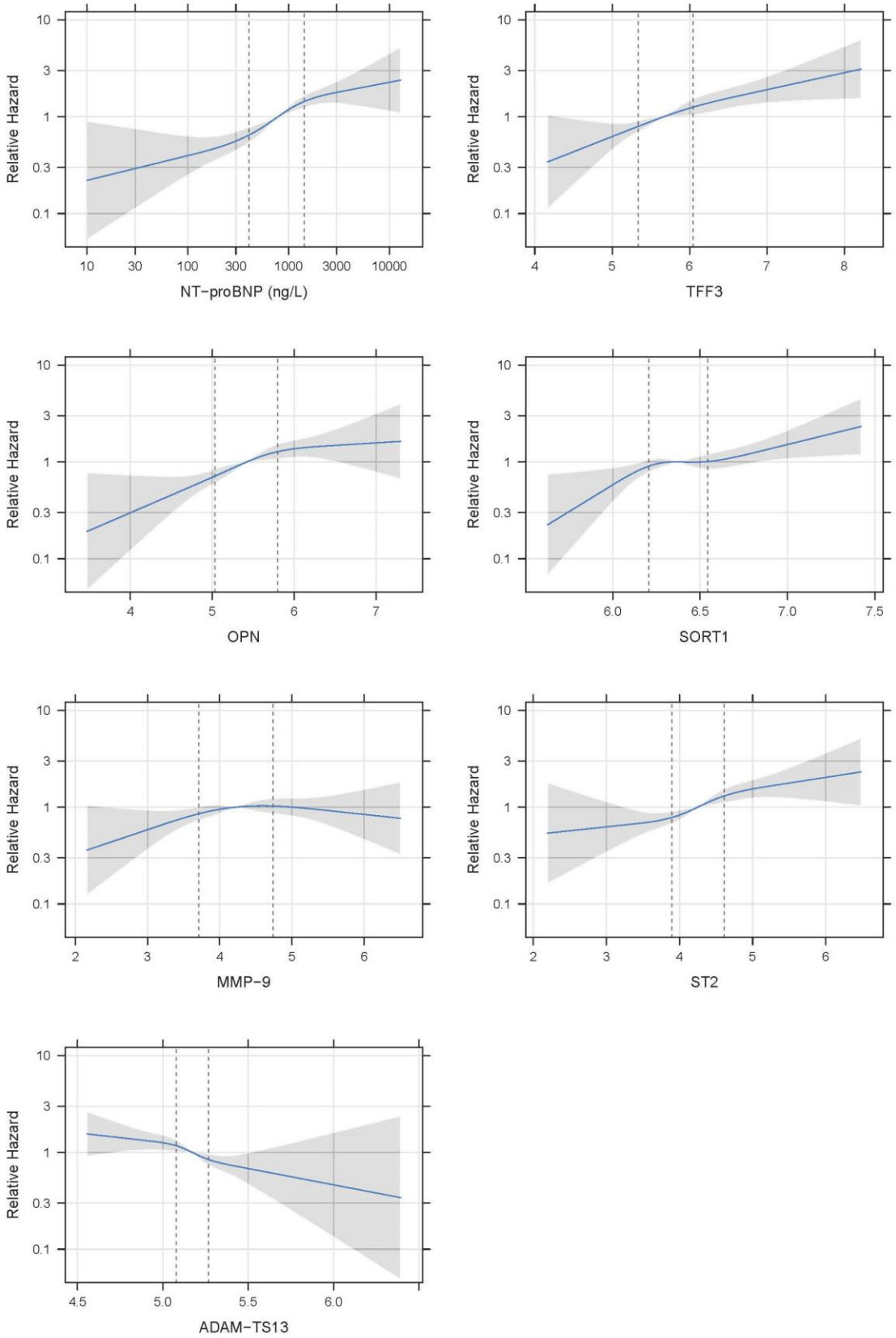
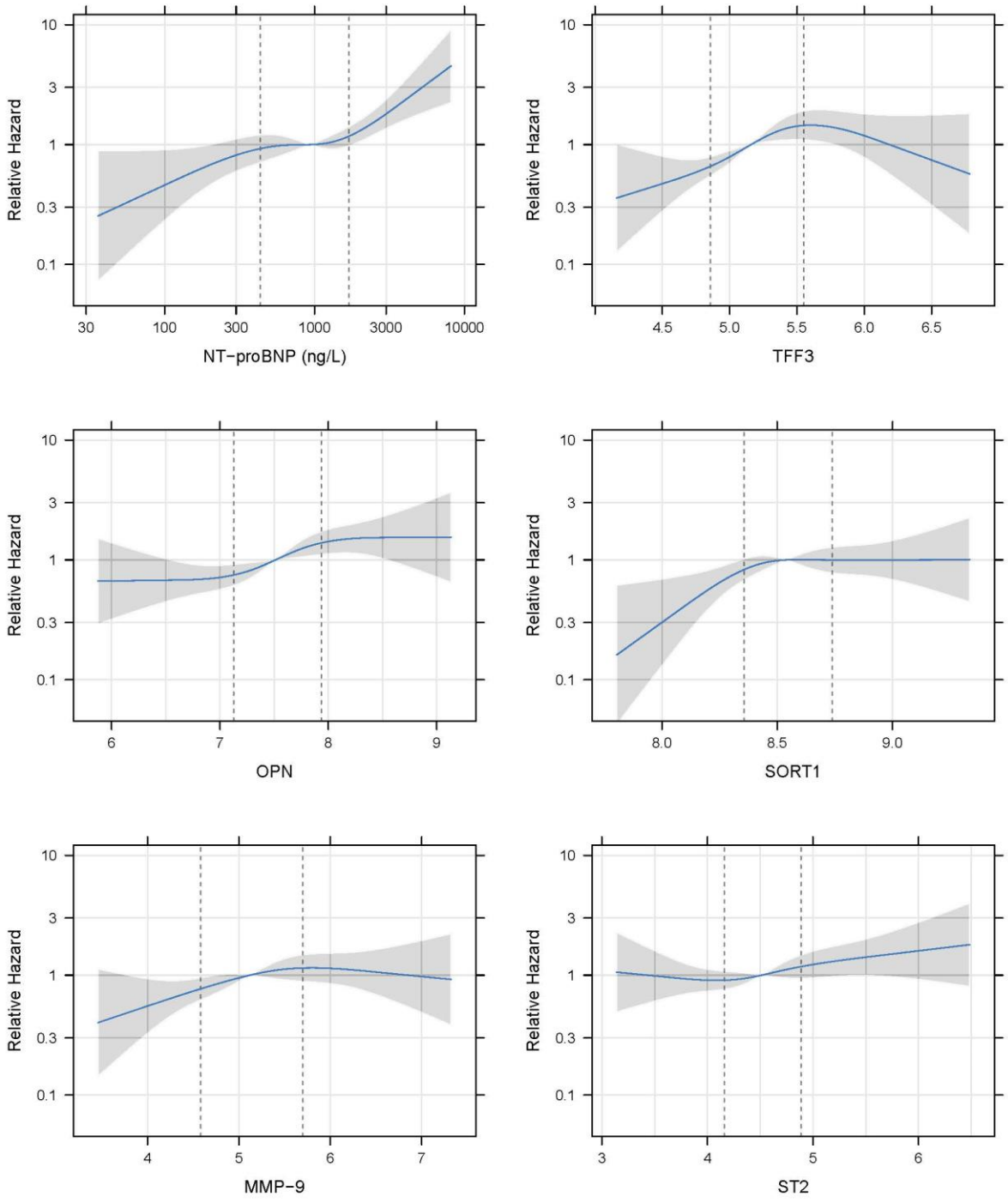


Figure S2B

Association of the top biomarkers (Table 3) with ischemic stroke/systemic embolism by using splines in the validation cohort