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.2 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 as...
Hijazi et al Screening for Novel Stroke Biomarkers in AF

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

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

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

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.

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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. 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 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). Estimated glomerular filtration rate was calculated on the basis of centrally determined creatinine levels using the Chronic Kidney Disease Epidemiology Collaboration equation.

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<sup>96</sup>x<sup>96</sup>, CVD III<sup>96</sup>x<sup>96</sup>, and Inflammation<sup>96</sup>x<sup>96</sup> 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. 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%. Prior validation studies have also showed that biomarkers analyzed with the PEA technique have an adequate concordance with conventional immunoassays. 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\textsuperscript{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.\textsuperscript{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,\textsuperscript{17} using the ranger\textsuperscript{18} package.

**RESULTS**

Baseline Characteristics and Distribution of Biomarkers

The baseline characteristics of the multimarker sub-study 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
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 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 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 analyses, 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, cTn-hs, and cTnT-hs) and renal biomarkers (cystatin C) is shown in Table 4. TFF3 was moderately correlated with renal function (p = 0.59). Beyond that, no strong patterns of correlation were seen (p < 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, 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 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; 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.
| Biomarker | Low (ng/L) | High (ng/L) | HR [95% CI] | P   |
|----------|------------|-------------|--------------|-----|
| A        |            |             |              |     |
| NT-proBNP | 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, 1.549] | 5.748e−03 |
| vWF      | 5.854      | 7.059       | 1.285 [1.091, 1.514] | 2.638e−03 |
| cTnT-hs | 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] | 7.618e−03 |
| RARRES2  | 11.88      | 12.23       | 1.265 [1.039, 1.539] | 9.101e−02 |
| PAi      | 5.591      | 6.889       | 1.261 [1.050, 1.514] | 1.291e−02 |
| TGM2     | 7.805      | 8.776       | 1.245 [1.038, 1.483] | 3.816e−02 |
| BLM hydrolase | 5.055   | 6.066       | 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        | 1.231 [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, 1.360] | 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] | 3.816e−03 |
| PI3      | 3.786      | 4.68        | 1.218 [1.004, 1.436] | 4.428e−02 |
| CASP−8   | 2.38       | 3.53        | 1.176 [1.016, 1.361] | 2.992e−02 |
| AXL      | 7.925      | 8.397       | 1.175 [0.999, 1.363] | 5.159e−02 |
| MMP−9    | 3.711      | 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 |
| GDF−1    | 4.936      | 6.204       | 0.860 [0.743, 0.979] | 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 |

### A Hazard Ratio Analysis

- **NT-proBNP** (ng/L)
- **Low**: 435
- **High**: 1696
- **HR [95% CI]**
- **P**: 0.00633

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- **P**: 0.00633
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 3. Biomarkers Consistently Associated With Ischemic Stroke/SE, According to RF or Adjusted Cox Regression Analyses

| Variable            | RF Ranking | Low  | High   | 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)   | 1.0036E-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 (1.231–1.633)    | 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 (1.342–2.248)    | 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<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.
Osteopontin has also been suggested with higher concentrations in the presence of cardiac fibrosis,27,28 osteopontin has been described to be a mediator of cardiac fibrosis, 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 fibrosis30 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...
TFF3, a member of the trefoil family, is mainly secreted from goblet cells in the gastrointestinal system and is involved in supporting mucosal integrity. 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. Some studies have demonstrated increased concentrations in states of myocardial ischemia, and TFF3 has also been associated with cardioprotective effects in experimental animal models. 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. 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.\(^4\) 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 spindlin-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.\(^1\)

**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                   | Q9BYF1      | 3.9 (3.5 -- 4.4) [203] | 4.0 (3.6 -- 4.5) [12] | 1.1  |
| ADAM-TS13              | Q76LX8      | 5.2 (5.1 -- 5.3) [203] | 5.2 (5.1 -- 5.2) [12] | 1.4  |
| ADM                    | P35318      | 7.5 (7.2 -- 7.8) [203] | 7.6 (7.3 -- 7.9) [12] | 1.9  |
| AGRP                   | O00253      | 3.2 (3.0 -- 3.5) [203] | 3.3 (3.0 -- 3.6) [12] | 0.7  |
| AMBP                   | P02760      | 7.1 (7.0 -- 7.2) [203] | 7.1 (7.0 -- 7.3) [12] | 1.3  |
| ANG-1                  | Q15389      | 8.9 (8.1 -- 9.6) [203] | 9.0 (8.1 -- 9.8) [12] | 2.2  |
| BMP-6                  | P22004      | 5.7 (5.4 -- 6.0) [203] | 5.8 (5.5 -- 6.1) [12] | 1.4  |
| BNP                    | P16860      | 4.0 (3.0 -- 4.9) [203] | 4.5 (3.7 -- 5.4) [12] | 1.6  |
| CASA                   | P35218      | 2.6 (2.1 -- 3.3) [203] | 2.7 (2.2 -- 3.3) [12] | 1.3  |
| CCL17                  | Q92583      | 6.9 (6.3 -- 7.6) [203] | 6.9 (6.3 -- 7.7) [12] | 1.7  |
| CCL3                   | P10147      | 2.8 (2.5 -- 3.1) [203] | 2.9 (2.6 -- 3.2) [12] | 1.6  |
| CD4                    | P01730      | 4.7 (4.5 -- 4.9) [203] | 4.7 (4.5 -- 5.0) [12] | 1.3  |
| CD40-L                 | P29965      | 4.1 (3.5 -- 5.1) [203] | 4.2 (3.6 -- 5.1) [12] | 1.2  |
| CD84                   | Q0UIB8      | 5.3 (5.1 -- 5.6) [203] | 5.4 (5.1 -- 5.7) [12] | 1.4  |
| CEACAM8                | P31997      | 4.2 (3.9 -- 4.7) [203] | 4.3 (3.9 -- 4.7) [12] | 1.6  |
| CTRC                   | Q99895      | 10.2 (9.7 -- 10.7) [203] | 10.1 (9.7 -- 10.6) [12] | 2.6  |
| CTSL1                  | P07711      | 6.1 (5.7 -- 6.4) [203] | 6.1 (5.9 -- 6.6) [12] | 0.9  |
| CXCL1                  | P09341      | 8.3 (7.4 -- 8.9) [203] | 8.2 (7.5 -- 8.9) [12] | 2.7  |
| DCN                    | P07585      | 5.4 (5.3 -- 5.6) [203] | 5.5 (5.3 -- 5.6) [12] | 3.6  |
| DECR1                  | Q16698      | 3.1 (2.5 -- 3.8) [203] | 3.1 (2.5 -- 4.0) [12] | 2.0  |
| Dkk-1                  | O94907      | 8.9 (8.5 -- 9.3) [203] | 9.0 (8.6 -- 9.4) [12] | 2.1  |
| FABP2                  | P12104      | 9.2 (8.6 -- 9.7) [203] | 9.2 (8.6 -- 9.7) [12] | 2.1  |
| FGF-21                 | Q9NSA1      | 7.7 (6.8 -- 8.6) [203] | 7.9 (7.0 -- 8.8) [12] | 2.6  |
| Protein                  | Description                                      | Accession | Median Value [Range] | Min. Value [Range] | Max. Value [Range] | Ratio |
|-------------------------|--------------------------------------------------|-----------|----------------------|-------------------|-------------------|-------|
| FGF-23                  | Fibroblast growth factor 23                      | Q9GZV9    | 4.3 (3.8 -- 4.9)     | 4.3 (4.0 -- 4.9)  | 1.4               |
| FS                      | Follistatin                                       | P19883    | 12.0 (11.7 -- 12.3)  | 12.0 (11.8 -- 12.3)| 3.6               |
| Gal-9                   | Galectin-9                                       | O00182    | 7.0 (6.8 -- 7.2)     | 7.0 (6.8 -- 7.2)  | 1.5               |
| GDF-2                   | Growth/differentiation factor 2                  | Q9UK05    | 4.6 (4.2 -- 4.9)     | 4.6 (4.2 -- 5.0)  | 1.5               |
| GH                      | Growth hormone                                   | P01241    | 8.5 (7.0 -- 10.1)    | 8.9 (7.6 -- 10.1) | 1.8               |
| GIF                     | Gastric intrinsic factor                         | P27352    | 5.6 (5.0 -- 6.2)     | 5.5 (4.8 -- 6.4)  | 1.4               |
| GLO1                    | Lactoylglutathione lyase                         | Q04760    | 6.1 (5.7 -- 6.7)     | 6.2 (5.7 -- 6.7)  | 1.9               |
| GT                      | Gastrotropin                                      | P51161    | 1.9 (1.6 -- 2.4)     | 1.9 (1.6 -- 2.4)  | 1.2               |
| HAOX1                   | Hydroxyacid oxidase 1                            | Q9UJM8    | 4.6 (3.7 -- 5.7)     | 4.7 (3.7 -- 5.7)  | 1.1               |
| HB-EGF                  | Proheparin-binding EGF-like growth factor        | Q99075    | 5.8 (5.6 -- 6.1)     | 5.9 (5.6 -- 6.1)  | 0.8               |
| HO-1                    | Heme oxygenase 1                                 | P09601    | 11.7 (11.4 -- 11.9)  | 11.6 (11.4 -- 12.0)| 4.4               |
| hOSCAR                  | Osteoclast-associated immunoglobulin-like receptor| Q8IY55   | 9.8 (9.6 -- 9.9)     | 9.8 (9.6 -- 9.9)  | 3.0               |
| HSP 27                  | Heat shock 27 kDa protein                        | P04792    | 10.2 (9.6 -- 10.6)   | 10.2 (9.5 -- 10.6)| 2.4               |
| IDUA                    | Alpha-L-iduronidase                              | P35475    | 4.6 (4.1 -- 5.0)     | 4.5 (4.1 -- 4.9)  | 1.6               |
| Ig G Fc receptor II-b   | Low affinity immunoglobulin gamma Fc region receptor II-b | P31994 | 1.7 (1.4 -- 2.1)     | 1.8 (1.4 -- 2.1)  | 1.3               |
| IL-17D                  | Interleukin-17D                                  | Q8TAD2    | 2.7 (2.6 -- 2.9)     | 2.8 (2.6 -- 2.9)  | 1.2               |
| IL-18                   | Interleukin-18                                   | Q14116    | 8.7 (8.4 -- 9.1)     | 8.8 (8.3 -- 9.2)  | 2.5               |
| IL-1ra                  | Interleukin-1 receptor antagonist protein        | P18510    | 4.5 (4.1 -- 5.0)     | 4.7 (4.2 -- 5.1)  | 2.4               |
| IL-4RA                  | Interleukin-4 receptor subunit alpha             | P24394    | 3.2 (2.9 -- 3.5)     | 3.3 (3.0 -- 3.6)  | 1.2               |
| IL-6                    | Interleukin-6                                    | P05231    | 4.0 (3.5 -- 4.6)     | 4.1 (3.6 -- 4.5)  | 1.3               |
| IL16                    | Pro-interleukin-16                               | Q14005    | 5.3 (5.0 -- 5.6)     | 5.3 (5.0 -- 5.7)  | 1.0               |
| IL1RL2                  | Interleukin-1 receptor-like 2                    | Q9HB29    | 4.6 (4.3 -- 4.9)     | 4.7 (4.3 -- 4.9)  | 1.5               |
| IL-27                   | Interleukin-27                                   | Q8NEV9,   | 4.5 (4.2 -- 4.7)     | 4.5 (4.3 -- 4.8)  | 1.9               |
| ITGB1BP2                | Melusin                                          | Q9UKP3    | 3.0 (3.0 -- 4.5)     | 3.1 (3.0 -- 4.8)  | 3.0               |
| LEP                     | Leptin                                           | P41159    | 6.7 (6.0 -- 7.4)     | 6.7 (5.9 -- 7.3)  | 2.2               |
| LOX-1                   | Lectin-like oxidized LDL receptor 1              | P78380    | 6.9 (6.5 -- 7.3)     | 6.9 (6.6 -- 7.4)  | 2.7               |
| LPL                     | Lipoprotein lipase                               | P06858    | 9.5 (9.2 -- 9.8)     | 9.6 (9.3 -- 9.8)  | 2.7               |
| Gene Symbol | Protein Name | Uniprot ID | Fold Change | Standard Deviation | P-Value | Gene Name |
|-------------|--------------|------------|-------------|--------------------|---------|-----------|
| MARCO       | Macrophage receptor MARCO | Q9UEW3 | 6.2 (6.1 -- 6.4) | [203] | 1.3 |
| MERTK       | Tyrosine-protein kinase Mer | Q12866 | 4.5 (4.2 -- 4.7) | [203] | 1.6 |
| MMP-12      | Matrix metalloproteinase-12 | P39900 | 7.8 (7.3 -- 8.3) | [203] | 1.9 |
| MMP-7       | Matrix metalloproteinase-7 | P09237 | 7.3 (6.3 -- 8.1) | [203] | 3.3 |
| NEMO        | NF-kappa-B essential modulator | Q9Y6K9 | 4.2 (3.7 -- 5.0) | [203] | 1.9 |
| PAPPA       | Pappalysin-1 | Q13219 | 3.2 (2.9 -- 3.6) | [203] | 1.5 |
| PAR-1       | Proteinase-activated receptor 1 | P25116 | 7.4 (7.1 -- 7.7) | [203] | 1.8 |
| PARP-1      | Poly [ADP-ribose] polymerase 1 | P09874 | 3.4 (3.0 -- 3.8) | [203] | 1.9 |
| PD-L2       | Programmed cell death 1 ligand 2 | Q9BQ51 | 3.1 (2.9 -- 3.3) | [203] | 1.9 |
| PDGF subunit B | Platelet-derived growth factor subunit B | P01127 | 8.9 (8.1 -- 9.6) | [203] | 2.7 |
| PlgR        | Polymeric immunoglobulin receptor | P01833 | 6.5 (6.4 -- 6.5) | [203] | 1.8 |
| PIGF        | Placenta growth factor | P49763 | 8.1 (7.9 -- 8.3) | [203] | 1.5 |
| PRELP       | Prolargin | P51888 | 6.7 (6.6 -- 6.8) | [203] | 1.5 |
| Protein BOC | Brother of CDO | Q9BWW1 | 4.9 (4.7 -- 5.0) | [203] | 1.2 |
| PRSS27      | Serine protease 27 | Q9BQR3 | 8.1 (7.8 -- 8.4) | [203] | 2.0 |
| PRSS58      | Prostasin | Q16651 | 9.2 (9.0 -- 9.5) | [203] | 1.4 |
| PSGL-1      | P-selectin glycoprotein ligand 1 | Q14242 | 5.0 (4.8 -- 5.1) | [203] | 1.3 |
| PTX3        | Pentraxin-related protein PTX3 | P26022 | 3.7 (3.4 -- 4.0) | [203] | 1.7 |
| RAGE        | Receptor for advanced glycosylation end products | Q15109 | 5.3 (5.0 -- 5.6) | [203] | 1.1 |
| REN         | Renin | P00797 | 7.5 (6.8 -- 8.1) | [203] | 2.0 |
| SCF         | Stem cell factor | P21583 | 9.7 (9.4 -- 9.9) | [203] | 2.6 |
| SERPINA12   | Serpin A12 | Q8I7W75 | 3.6 (3.0 -- 4.4) | [203] | 3.0 |
| SLAMF7      | SLAM family member 7 | Q9NQ25 | 2.4 (2.4 -- 2.8) | [203] | 2.4 |
| SOD2        | Superoxide dismutase [Mn], mitochondrial | P04179 | 8.9 (8.7 -- 9.0) | [203] | 1.8 |
| SORT1       | Sortilin | Q99523 | 6.4 (6.2 -- 6.5) | [203] | 1.6 |
| SPON2       | Spondin-2 | Q9BUD6 | 9.0 (8.9 -- 9.1) | [255] | 2.1 |
| SRC         | Proto-oncogene tyrosine-protein kinase Src | P12931 | 5.5 (4.4 -- 7.0) | [203] | 1.4 |
| STK4        | Serine/threonine-protein kinase 4 | Q13043 | 1.9 (1.2 -- 3.5) | [203] | 1.2 |
| TF          | Tissue factor | P13726 | 5.8 (5.6 -- 6.0) | [203] | 1.3 |
| Gene Symbol | Gene Name | Description | NCBI ID | Gene Symbol | Gene Name | Description | NCBI ID |
|-------------|-----------|-------------|---------|-------------|-----------|-------------|---------|
| 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 | Q14763 | 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 | Q15467 | 6.1 (5.8 -- 6.5) [23] | 6.3 (6.0 -- 6.6) [0] | 0.4 |
| CCL22       | C-C motif chemokine 22 | Q00175 | 2.3 (2.0 -- 2.8) [23] | 2.4 (2.0 -- 2.7) [0] | 1.3 |
| CCL24       | C-C motif chemokine 24 | Q00175 | 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 |
| Gene  | Description                                                                 | Accession | Expression | Log2 Fold Change | p Value |
|-------|-----------------------------------------------------------------------------|-----------|------------|-----------------|---------|
| CHI3L1| Chitinase-3-like protein 1                                                   | P36222    | 7.4        | 6.8 -- 8.2 [23] | 3.3     |
| CHIT1 | Chitotriosidase-1                                                           | Q13231    | 3.4        | 2.6 -- 4.1 [23] | -1.2    |
| CNTN1 | Contactin-1                                                                 | Q12860    | 3.2        | 3.0 -- 3.4 [23] | 0.5     |
| COL1A1| Collagen alpha-1(l) chain                                                   | P02452    | 2.5        | 2.2 -- 2.8 [23] | -0.1    |
| CPA1  | Carboxypeptidase A1                                                         | P15085    | 4.6        | 4.2 -- 5.1 [23] | 0.9     |
| CPB1  | Carboxypeptidase B                                                          | P15086    | 4.1        | 3.7 -- 4.6 [23] | 0.6     |
| CSTB  | Cystatin-B                                                                  | P04080    | 5.1        | 4.8 -- 5.6 [93] | 4.1     |
| CTSZ  | Cathepsin Z                                                                 | P07339    | 4.3        | 4.0 -- 4.7 [23] | 3.5     |
| CXCL16| C-X-C motif chemokine 16                                                    | Q9H2A7    | 6.3        | 6.1 -- 6.5 [23] | 0.7     |
| DK1   | Protein delta homolog 1                                                     | P80370    | 5.4        | 5.0 -- 5.8 [23] | 0.7     |
| EGFR  | Epidermal growth factor receptor                                            | P00533    | 1.9        | 1.8 -- 2.1 [23] | -0.1    |
| Ep-CAM| Epithelial cell adhesion molecule                                           | P16422    | 4.0        | 3.5 -- 4.7 [23] | 0.9     |
| EPHB4 | Ephrin type-B receptor 4                                                    | P54760    | 2.3        | 2.1 -- 2.5 [23] | 1.4     |
| FABP4 | Fatty acid-binding protein, adipocyte                                       | P15090    | 5.1        | 4.5 -- 5.7 [23] | 1.2     |
| FAS   | Tumor necrosis factor receptor superfamily member 6                         | P25445    | 5.0        | 4.8 -- 5.2 [23] | 1.3     |
| Gal-3 | Galectin-3                                                                  | P17931    | 5.7        | 5.4 -- 5.9 [23] | 4.0     |
| Gal-4 | Galectin-4                                                                  | P56470    | 3.8        | 3.4 -- 4.2 [23] | 0.9     |
| GDF-15| Growth/differentiation factor 15                                             | Q99988    | 5.3        | 4.9 -- 5.8 [23] | 1.9     |
| GRN   | Granulins                                                                   | P28799    | 3.5        | 3.3 -- 3.7 [23] | -0.5    |
| ICAM-2| Intercellular adhesion molecule 2                                            | P13598    | 5.3        | 5.0 -- 5.5 [23] | 1.1     |
| IGFBP-1| Insulin-like growth factor-binding protein 1                                | P08833    | 5.4        | 4.4 -- 6.2 [23] | 1.4     |
| IGFBP-2| Insulin-like Growth Factor-Binding Protein 2                                | P18065    | 8.1        | 7.5 -- 8.5 [23] | 1.2     |
| IGFBP-7| Insulin-like growth factor-binding protein 7                                 | Q16270    | 4.6        | 4.3 -- 4.9 [23] | 0.8     |
| IL-17RA| Interleukin-17 receptor A                                                    | Q96F46    | 4.2        | 3.8 -- 4.5 [23] | 1.5     |
| IL-18BP| Interleukin-18-binding protein                                               | Q95998    | 6.7        | 6.5 -- 7.0 [23] | 1.2     |
| IL-1RT1| Interleukin-1 receptor type 1                                               | P14778    | 6.6        | 6.4 -- 6.8 [23] | 1.7     |
| IL-1RT2| Interleukin-1 receptor type 2                                               | P27930    | 5.2        | 5.0 -- 5.5 [23] | 1.8     |
| IL-6RA| Interleukin-6 receptor subunit alpha                                         | P08887    | 11.0       | 10.7 -- 11.3 [23]| 4.0     |
| Gene Symbol | Protein Name                           | Entrez ID | Value               | Value Range | p | Ratio |
|-------------|----------------------------------------|-----------|---------------------|-------------|---|-------|
| 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        | Lymphoxygen-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 -- 3.4) [0]| 1.4 |
| PDGF subunit A | Platelet-derived growth factor subunit A | P04085 | 3.1 (3.4 -- 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     | Q75594    | 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 |
| Gene Symbol | Description | Entrez ID | Mean (95% CI) [ref] | Mean (95% CI) [ref] | Mean (95% CI) | Mean (95% CI) | | |
|-------------|-------------|-----------|---------------------|---------------------|---------------|---------------|-----------|
| SCGB3A2     | Secretoglobin family 3A member 2 | Q96PL1    | 2.6 (2.2 -- 3.2) [23] | 2.7 (2.3 -- 3.3) [0] | 0.4           | 0.4           | |
| SELE        | E-selectin  | P16581    | 2.5 (2.1 -- 2.8) [23] | 2.5 (2.2 -- 2.8) [0] | 0.7           | 0.7           | |
| SELP        | P-selectin  | P16109    | 9.1 (8.7 -- 9.5) [23] | 9.1 (8.7 -- 9.6) [0] | 2.5           | 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           | 1.5           | |
| SPON1       | Spondin-1   | Q9HCB6    | 2.3 (2.1 -- 2.5) [23] | 2.4 (2.2 -- 2.6) [0] | 1.4           | 1.4           | |
| ST2         | ST2 protein | Q01638    | 4.2 (3.9 -- 4.6) [23] | 4.4 (4.0 -- 4.7) [0] | 1.5           | 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           | 1.1           | |
| TFF3        | Trefoil factor 3 | Q07654    | 5.6 (5.3 -- 6.0) [23] | 5.7 (5.4 -- 6.2) [0] | 2.9           | 2.9           | |
| TFPI        | Tissue factor pathway inhibitor | P10646   | 8.6 (8.3 -- 8.8) [23] | 8.6 (8.4 -- 8.9) [0] | 1.7           | 1.7           | |
| TIMP4       | Metalloproteinase inhibitor 4 | Q99727   | 5.2 (4.8 -- 5.5) [23] | 5.3 (5.0 -- 5.7) [0] | 0.7           | 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           | 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           | 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           | 1.1           | |
| TNFRSF10C   | Tumor necrosis factor receptor superfamily member 10C | Q14798 | 6.1 (5.8 -- 6.4) [23] | 6.0 (5.7 -- 6.4) [0] | 1.5           | 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           | 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           | 1.6           | |
| TR          | Transferrin receptor protein 1 | Q01638    | 5.2 (4.8 -- 5.6) [23] | 5.3 (4.9 -- 5.7) [0] | 1.2           | 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           | 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           | 1.9           | |
| uPA         | Urokinase-type plasminogen activator | P00749 | 5.0 (4.8 -- 5.2) [23] | 5.0 (4.8 -- 5.3) [0] | 1.0           | 1.0           | |
| vWF         | von Willebrand factor | P04275 | 6.4 (5.8 -- 7.0) [23] | 6.6 (6.1 -- 7.3) [0] | 1.4           | 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           | 1.4           | |
| ADA         | Adenosine Deaminase | P00813 | 4.1 (3.9 -- 4.4) [188] | 4.1 (3.9 -- 4.4) [18] | 0.3           | 0.3           | |
| ARTN        | Artemin | QST4W7   | 0.2 (0.2 -- 0.2) [188] | 0.2 (0.2 -- 0.2) [18] | 0.2           | 0.2           | |
| AXIN1       | Axin-1 | O15169 | 1.6 (1.4 -- 2.5) [188] | 1.6 (1.4 -- 2.7) [18] | 1.4           | 1.4           | |
| BDNF        | Brain-derived neurotrophic factor | P23560 | 2.4 (2.4 -- 6.2) [188] | 2.4 (2.4 -- 5.9) [18] | 2.4           | 2.4           | |
| Protein | Description | Accession | Ratio | Lower Limit | Upper Limit | P01138 Ratio | P01138 Lower Limit | P01138 Upper Limit |
|---------|-------------|-----------|-------|--------------|-------------|---------------|-------------------|-------------------|
| Beta-NGF | Beta-nerve growth factor | P01138 | 1.6 (1.4 -- 1.8) | 1.6 (1.5 -- 1.8) | 0.8 |
| CASP-8 | Caspase 8 | Q14790 | 2.8 (2.4 -- 3.5) | 3.0 (2.4 -- 3.7) | 1.0 |
| CCL11 | Eotaxin-1 | P51671 | 8.1 (7.9 -- 8.4) | 8.2 (7.9 -- 8.5) | 1.3 |
| CCL19 | C-C motif chemokine 19 | Q99731 | 9.4 (9.0 -- 10.0) | 9.6 (9.0 -- 10.1) | 1.4 |
| CCL20 | C-C motif chemokine 20 | P78556 | 6.6 (6.1 -- 7.3) | 6.8 (6.1 -- 7.5) | 1.8 |
| CCL23 | C-C motif chemokine 23 | P55773 | 10.0 (9.7 -- 10.4) | 10.1 (9.9 -- 10.4) | 1.1 |
| CCL25 | C-C motif chemokine 25 | Q15444 | 6.9 (6.5 -- 7.3) | 6.9 (6.5 -- 7.3) | 1.0 |
| CCL28 | C-C motif chemokine 28 | Q9NRJ3 | 1.0 (0.8 -- 1.2) | 1.1 (0.9 -- 1.3) | 0.1 |
| CCL4 | C-C motif chemokine 4 | P13236 | 5.8 (5.5 -- 6.2) | 5.9 (5.5 -- 6.3) | 0.3 |
| CD244 | Natural killer cell receptor 2B4 | Q9BZW8 | 6.0 (5.8 -- 6.2) | 6.0 (5.8 -- 6.3) | 1.7 |
| CD40 | CD40L receptor | P25942 | 9.3 (9.1 -- 9.6) | 9.4 (9.2 -- 9.7) | 1.3 |
| CD5 | T-cell surface glycoprotein CD5 | P06127 | 3.4 (3.2 -- 3.7) | 3.5 (3.3 -- 3.8) | -0.4 |
| CD6 | T cell surface glycoprotein CD6 isoform | Q8WWJ7 | 4.0 (3.7 -- 4.2) | 4.0 (3.7 -- 4.3) | 0.8 |
| CDCP1 | CUB domain-containing protein 1 | Q9HSV8 | 3.0 (2.6 -- 3.4) | 3.1 (2.7 -- 3.6) | 0.0 |
| CSF-1 | Macrophage colony-stimulating factor 1 | P09603 | 8.0 (7.8 -- 8.1) | 8.0 (7.9 -- 8.1) | 0.6 |
| CST5 | Cystatin D | P28325 | 7.0 (6.6 -- 7.4) | 7.0 (6.6 -- 7.4) | 3.2 |
| CX3CL1 | Fractalkine | P78423 | 5.8 (5.5 -- 6.0) | 5.8 (5.5 -- 6.2) | 1.6 |
| CXCL10 | C-X-C motif chemokine 10 | P02778 | 10.3 (9.8 -- 10.8) | 10.4 (10.0 -- 11.0) | 2.0 |
| CXCL11 | C-X-C motif chemokine 11 | O14625 | 6.9 (6.4 -- 7.5) | 7.0 (6.4 -- 7.7) | 1.7 |
| CXCL5 | C-X-C motif chemokine 5 | P42830 | 10.7 (9.5 -- 11.7) | 10.8 (9.6 -- 11.8) | 3.7 |
| CXCL6 | C-X-C motif chemokine 6 | P80162 | 7.3 (6.8 -- 7.9) | 7.4 (6.9 -- 7.9) | 1.3 |
| CXCL9 | C-X-C motif chemokine 9 | Q07325 | 8.5 (8.0 -- 9.1) | 8.6 (8.1 -- 9.2) | 1.9 |
| DNER | Delta and Notch-like epidermal growth factor-related receptor | Q8NFT8 | 7.1 (6.9 -- 7.3) | 7.1 (6.9 -- 7.3) | 0.6 |
| EN-RAGE | Protein S100-A12 | P80511 | 2.7 (2.1 -- 3.2) | 2.8 (2.3 -- 3.3) | 0.7 |
| FGF-19 | Fibroblast growth factor 19 | O95750 | 8.1 (7.4 -- 8.8) | 8.2 (7.4 -- 8.9) | 1.1 |
| FGF-5 | Fibroblast growth factor 5 | Q8NF90 | 1.9 (1.7 -- 2.1) | 1.9 (1.8 -- 2.1) | 1.4 |
| Flt3L | Fms-related tyrosine kinase 3 ligand | P49771 | 9.3 (9.1 -- 9.6) | 9.4 (9.1 -- 9.6) | 1.8 |
| hGDNF | Glial cell line-derived neurotrophic factor | P39905 | 2.2 (2.0 -- 2.5) | 2.3 (2.0 -- 2.5) | 1.5 |
| Protein                          | Gene      | ID          | Regulation | P14210 | 7.5 (7.2 -- 7.8) | 7.6 (7.4 -- 7.9) | 0.8                   |
|---------------------------------|-----------|-------------|------------|--------|-----------------|-----------------|-----------------------|
| IFN-gamma                       | P01579    | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7                   |
| IL-1 alpha                      | P01583    | IL-10       | 4.3 (4.0 -- 4.6) | 4.3 (4.0 -- 4.6) | 2.1 (2.1 -- 2.1) | 2.1 (2.1 -- 2.1) | 2.1 (2.1 -- 2.1) | 2.1                   |
| IL-10                           | P22301    | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9                   |
| IL-10RA                         | Q13651    | 6.7 (6.5 -- 6.9) | 6.7 (6.5 -- 7.0) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8                   |
| IL-12B                          | P29460    | 4.8 (4.4 -- 5.3) | 5.0 (4.5 -- 5.4) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 0.9                   |
| IL-13                           | P35225    | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.1 (1.1 -- 1.1) | 1.1                   |
| IL-15RA                         | Q13261    | 1.1 (0.9 -- 1.3) | 1.1 (0.9 -- 1.3) | 0.5 (0.5 -- 0.5) | 0.5 (0.5 -- 0.5) | 0.5 (0.5 -- 0.5) | 0.5 (0.5 -- 0.5) | 0.5                   |
| IL-17A                          | Q16552    | 0.5 (0.4 -- 0.8) | 0.5 (0.4 -- 0.8) | 1.5 (1.5 -- 1.8) | 1.5 (1.5 -- 1.8) | 1.5 (1.5 -- 1.8) | 1.5 (1.5 -- 1.8) | 1.5                   |
| IL-17C                          | Q9P0M4    | Q9UHF4      | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3                   |
| IL-18R1                         | Q13478    | 7.5 (7.2 -- 7.8) | 7.6 (7.3 -- 7.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8                   |
| IL-2                            | P60568    | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4                   |
| IL-20                           | Q9NYY1    | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 1.5 (1.5 -- 1.5) | 1.5 (1.5 -- 1.5) | 1.5 (1.5 -- 1.5) | 1.5 (1.5 -- 1.5) | 1.5                   |
| IL-20RA                         | Q9UHF4    | 0.9 (0.9 -- 0.9) | 0.9 (0.9 -- 0.9) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3                   |
| IL-24                           | Q13007    | 0.4 (0.4 -- 0.4) | 0.4 (0.4 -- 0.4) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7                   |
| IL-22 RA1                       | Q8N6P7    | 1.6 (1.6 -- 2.0) | 1.6 (1.6 -- 2.0) | 3.3 (2.8 -- 3.8) | 3.3 (2.8 -- 3.8) | 3.3 (2.8 -- 3.8) | 3.3 (2.8 -- 3.8) | 3.3                   |
| LAP TGF-beta-1                  | P14784    | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7 (1.7 -- 1.7) | 1.7                   |
| LIF                             | P015112   | P01137      | 6.3 (6.1 -- 6.6) | 6.4 (6.1 -- 6.7) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8 (0.8 -- 0.8) | 0.8                   |
| LIF R                           | P015112   | P15018      | 0.6 (0.6 -- 0.6) | 0.6 (0.6 -- 0.6) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4 (1.4 -- 1.4) | 1.4                   |
| MCP-2                           | P42702    | 4.0 (3.8 -- 4.1) | 4.0 (3.8 -- 4.2) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3 (2.3 -- 2.3) | 2.3                   |
| MCP-3                           | P80075    | 9.1 (8.6 -- 9.5) | 9.1 (8.6 -- 9.5) | 5.1 (5.1 -- 5.1) | 5.1 (5.1 -- 5.1) | 5.1 (5.1 -- 5.1) | 5.1 (5.1 -- 5.1) | 5.1                   |
| MCP-3                           | P80098    | 2.1 (1.9 -- 2.4) | 2.2 (1.9 -- 2.5) | 1.9 (1.9 -- 2.1) | 1.9 (1.9 -- 2.1) | 1.9 (1.9 -- 2.1) | 1.9 (1.9 -- 2.1) | 1.9                   |
| Gene Symbol | Description | Accession | Median (Q1--Q3) [Reference] | Median (Q1--Q3) [Reference] | Difference |
|-------------|-------------|-----------|-----------------------------|-----------------------------|------------|
| 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       | Sulfrtransferase 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] | 0.0        |
| 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                      | Q9BYF1      | 4.4 (3.9 -- 4.8) | 4.4 (4.1 -- 5.0)  | 0.3   |
| ADAM-TS13                 | Q76LX8      | 5.9 (5.8 -- 6.0) | 5.9 (5.8 -- 6.0)  | 1.1   |
| ADM                       | P35318      | 7.3 (6.9 -- 7.6) | 7.3 (7.0 -- 7.7)  | 1.2   |
| AGRP                      | O00253      | 5.0 (4.7 -- 5.3) | 5.1 (4.8 -- 5.3)  | 0.8   |
| AMBP                      | P02760      | 7.5 (7.3 -- 7.6) | 7.5 (7.3 -- 7.7)  | 0.8   |
| ANGPT1                    | Q15389      | 8.0 (7.1 -- 8.8) | 8.2 (7.2 -- 9.1)  | 0.6   |
| BMP-6                     | P22004      | 4.8 (4.5 -- 5.1) | 4.9 (4.6 -- 5.2)  | 0.8   |
| BNP                       | P16860      | 5.4 (4.0 -- 6.4) | 5.7 (4.4 -- 6.8)  | 1.5   |
| Protein BOC               | Q9BWV1      | 3.9 (3.7 -- 4.1) | 3.9 (3.7 -- 4.2)  | 1.0   |
| CASA                      | Q92583      | 2.2 (1.7 -- 2.9) | 2.3 (1.8 -- 3.0)  | 1.6   |
| CCL17                     | P10147      | 7.7 (7.1 -- 8.5) | 7.8 (7.4 -- 8.7)  | 1.3   |
| CCL3                      | P01730      | 6.3 (5.9 -- 6.7) | 6.4 (6.1 -- 6.9)  | 1.2   |
| CD4                       | P29965      | 5.3 (5.1 -- 5.5) | 5.4 (5.2 -- 5.6)  | 0.7   |
| CD40-L                    | Q9UIB8      | 3.6 (3.0 -- 4.6) | 3.9 (3.3 -- 5.2)  | 0.8   |
| CD84                      | P31997      | 4.1 (3.8 -- 4.4) | 4.2 (3.9 -- 4.4)  | 1.6   |
| CEACAM8                   | Q99895      | 4.3 (3.9 -- 4.7) | 4.3 (4.0 -- 4.7)  | 1.7   |
| CTRC                      | P07711      | 10.2 (9.7 -- 10.7) | 10.2 (9.8 -- 10.8) | 1.9   |
| CTS1L                     | P09341      | 6.9 (6.7 -- 7.1) | 7.0 (6.7 -- 7.3)  | 0.9   |
| CXCL1                     | P07585      | 8.8 (7.9 -- 9.6) | 9.0 (8.0 -- 9.7)  | 2.1   |
| DCN                       | Q16698      | 4.9 (4.7 -- 5.1) | 5.0 (4.8 -- 5.2)  | 0.9   |
| DECR1                     | O94907      | 3.7 (3.1 -- 4.6) | 3.9 (3.2 -- 4.7)  | 1.7   |
| Dkk-1                     | P12104      | 8.4 (8.1 -- 8.9) | 8.6 (8.3 -- 9.1)  | 0.8   |
| FABP2                     | Q9NSA1      | 8.8 (8.2 -- 9.4) | 8.8 (8.3 -- 9.4)  | 1.5   |
| Protein Name       | Description                                                                 | GeneID | Mean       | Range     | Fold Change |
|-------------------|-------------------------------------------------------------------------------|--------|------------|-----------|-------------|
| 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         |
| GDF-2             | Galectin-9                                                                    | 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         |
| HBP-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                                                               | Q8IY55 | 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 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         |
| Gene  | Description                                      | UniProtID | Median (25% -- 75%) | 95% (90% -- 99%) | Fold Change |
|-------|--------------------------------------------------|-----------|---------------------|------------------|-------------|
| 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                 | Q9ISS51   | 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         |
| PIGF  | Placenta growth factor (PIGF)                    | 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)    | 8.2 (8.0 -- 8.3) | 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         |
| SERPINA2 | Serpin A12                                     | Q8H7W5    | 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)    | 4.0 (3.7 -- 4.4) | 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         |
| Gene Symbol | Gene Name                                      | Accession | Mean Log2 Expression | 90% CI       | Fold Change |
|------------|-----------------------------------------------|-----------|----------------------|--------------|-------------|
| 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       | Thrombopoi etin                               | 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 | Q86V87 | 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         |
| Gene    | Description                                      | UniProt ID | Mean Value | 95% Confidence Interval | Variance |
|---------|--------------------------------------------------|------------|------------|--------------------------|----------|
| 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      |
| CTD     | Cathepsin D                                      | P07339     | 2.4 (2.1 -- 2.7)  | 2.4 (2.1 -- 2.8)         | 0.1      |
| CTS2    | 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)  | 5.8 (5.6 -- 6.1)         | 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-1RA  | Interleukin-1 receptor A                         | Q96F46     | 4.0 (3.7 -- 4.3)  | 4.1 (3.7 -- 4.3)         | 1.6      |
| IL-18BP | Interleukin-18-binding protein                   | Q95998     | 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      |
| Gene     | Description                                      | UniProt ID | Mean   | Median | Standard Deviation |
|----------|--------------------------------------------------|------------|--------|--------|--------------------|
| 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    | Q8NB7      | 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                |
| PGLYR1   | Peptidoglycan recognition protein 1              | Q75594     | 7.3 (6.9 -- 7.6) | 7.4 (7.0 -- 7.7) | 0.4                |
| P33      | 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       | Q99699     | 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                |
| Protein Name       | Description                                                | Protein ID | Median (Q1-Q3)         | Lower (Q1-Q3)         | Upper (Q1-Q3)         | Ratio |
|-------------------|------------------------------------------------------------|------------|------------------------|------------------------|------------------------|-------|
| 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   |
| CTS1           | 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)  | 4.3 (3.7 -- 4.7)            | 0.0592  |
| OPN                   | 5.4 (5.0 -- 5.7)  | 5.6 (5.2 -- 5.9)            | 1.09E-08|
| SORT1                 | 6.4 (6.2 -- 6.5)  | 6.4 (6.2 -- 6.6)            | 1.05E-04|
| ST2                   | 4.2 (3.9 -- 4.6)  | 4.4 (4.0 -- 4.7)            | 1.44E-06|
| TFF3                  | 5.6 (5.3 -- 6.0)  | 5.7 (5.4 -- 6.2)            | 4.16E-09|

| Validation cohort     | No event (n=1,062) | Ischemic stroke/SE (n=149) | p-value |
|-----------------------|-------------------|-----------------------------|---------|
| 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
Figure S1B

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

| Protein | Hazard ratio (HR) [95% CI] | P value |
|---------|-----------------------------|---------|
| NT-proBNP (ng/L) | 1.237 [1.102, 1.389] | 0.002 |
| TIM | 1.322 [1.194, 1.457] | 0.0001 |
| CTSL1 | 1.408 [1.270, 1.559] | 0.0001 |
| TNFRSF10A | 1.551 [1.338, 1.806] | 0.0001 |
| OPN | 1.595 [1.351, 1.886] | 0.0001 |
| CT14 | 1.649 [1.413, 1.931] | 0.0001 |

Hazard ratio (HR) and 95% confidence interval (CI) for each biomarker are shown.
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